THE LARVAL DEVELOPMENT OF SOME COPEPODS OF THE FAMILY ASCIDICOLIDAE, SUBFAMILY HAPLOSTOMINAE, SYMBIONTS OF COMPOUND ASCIDIANS

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With Text-figures 1–9 and Table 1

Introduction

The copepods of the family Ascidicolidae, subfamily Haplostominae, exhibit a well-developed maxilliped, as do the Botryllophyilinae of the same family. However, in most of the haplostomins the adult females lack some mouthparts while in female botryllophilins the mouthparts are fully represented. The Haplostominae Chatton and Harant, 1924c, includes four genera, *Haplostomides, Haplostoma, Haplosaccus* and *Haplostomella,* in which the majority of species are symbionts of compound ascidians. *Haplostomides* Chatton and Harant, 1924b, has been the only genus known to possess all of the mouthparts. On the other hand it has been reported in their original papers that in *Haplostoma* Canu, 1886, and *Haplostomella* Chatton & Harant, 1924a, the mouthparts generally consist of two pairs (mandibles and maxilipeds) and in *Haplosaccus* Chatton and Brément, 1910, the mouthparts except for the maxillipeds are all absent. However, it seems that the terminology for the mouthparts used by previous authors for these three genera should be validated by ascertaining the definite developmental origin of the mouthparts.

There have been a few studies on the development of haplostomins. Canu (1892) was the first worker who succeeded in culturing and describing the larvae of *Haplostoma brevicauda* (Canu, 1886), from the first nauplius to the first copepodid. According to him the mouthparts of *Haplostoma* in the stages from first copepodid to adult female consist of two pairs, the mandibles and the second maxillae, although Dudley (1966, p. 160) pointed out that Canu considered the actual maxillipeds as the second maxillae.

In their embryological work, Anderson and Rossiter (1969) studied the general breeding activity, hatching and larval development of *Haplostomella australiensis.* They also state that the stages from first copepodid to adult female are provided with mandibles and maxillipeds, lacking the maxillules and maxillae.

However, neither of these embryological works have actually dealt directly with the origin of each of the mouthparts in the embryonic or larval stages.

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Two species of *Haplostomella, H. australiensis* Gotto, 1970 and *H. halocynthiae* (Fukui, 1965), are known as associates of simple ascidians. The terminology for the mouthparts of the female and larvae of *H. australiensis* does not correspond with that used in a redescription (Ooishi and Illg, 1974) of the mouthparts of the very similar species *H. halocynthiae*. In the redescription, it was pointed out that the terminology of the mouthparts of *Haplostomella,* as used by previous authors, is very questionable from the standpoint of morphological comparisons of the mouthparts of their species to those of the *Haplostomella* species from North America (Ooishi and Illg, 1977, unpublished at that time). In *H. halocynthiae* and the North American species the mouthparts are usually composed of three pairs of distinctly developed appendages: mandibles, maxillae and maxillipeds. In this case, the mandibles are definitely different in structure and position from those called "mandibles" in *H. australiensis* and most of the other known *Haplostomella* species.

During the taxonomic studies of the North American species (Ooishi and Illg, 1977) belonging to the other genera, a species of *Haplosaccus, H. elongatus* (female), has presented another problem, because its mouthparts (mandibles, maxillules, maxillae and maxillipeds) are fully present. Therefore, this species is in disagreement with *H. sacculus* of Chatton and Brément (1910) in regard to the complement of the mouthparts. Furthermore, it has been found that one species of *Haplostoma, H. elegans* (female), from North America is provided with three pairs of mouthparts (mandibles, maxillae and maxillipeds), thus differing from all other *Haplostoma* females including *H. brevicauda* of Canu. The same complement of the mouthparts of *H. elegans* has also been observed in the males of *Haplostoma* and *Haplostomella* from North America, but there was no previous description attributed to the males of the Haplostominae.

Consequently, to pursue the taxonomic studies on the North American species as well as *H. halocynthiae,* the terminology problem in regard to the mouthparts has been considered as a primary consideration, and it has been necessary to attempt to determine the origin of the mouthparts for all of the genera of the subfamily.

For this purpose embryos and larvae of many, though not all, of the species from North America were cultured. Of these, embryos or larvae of seven species, collected from compound ascidians, were cultured successfully, though not in all larval stages. These were *Haplosaccus elongatus* from *Amaroucium arenatum, Haplostoma albicatum* from *Distaplia occidentalis, Haplostoma elegans* from *Amaroucium propinquum, Haplostomella distincta* and *Haplostomella dubia* from *Amaroucium arenatum, Haplostomella oceanica* from *Eudistoma ritteri,* and *Haplostomella reducta* from *Distaplia occidentalis.*

The developmental studies on these copepod species were primarily undertaken to determine the origin of the mouthparts. In the process, some of the developmental aspects of representatives of the three genera, *Haplosaccus, Haplostoma* and *Haplostomella,* were brought out. These representatives included the first nauplius to first swimming copepodid stages of *Haplosaccus elongatus,* the first nauplius to first swimming copepodid stages and a later parasitic stage of *Haplosotma albicatum,* and

the first nauplius to probable fourth copepodid stages of *Haplostomella distincta.* In the description of the development of the latter two genera some of the larval stages, mainly including swimming and early or later parasitic copepodid stages, of *Haplostoma elegans, Haplostomella oceanica, H. dubia* and *H. reducta* are additionally presented. Although the larvae of the probable fourth and later stages were obtained directly from the host ascidians, all the other larvae were cultured in the laboratory. Among these larvae, those in the third and later copepodid stages were in infecting form. The invading stages of haplostomins have never previously been presented.

From the experience with the seven species and the compilation of the details of development, it is concluded that there are two developmental types (Types I, II) in this subfamily, indicated mainly by (1) characteristic periods of development for each type, (2) the pigmentation pattern of the nauplii and copepodids, and (3) the structure of the body and legs of the first copepodid.

The results of the studies of the morphogenesis from the naupliar appendages to the copepodid appendages and the recognition of the larval homologies of mouthparts in the adults provided the rationale for the terminology used for the mouthparts of the North American species and for *H. halocynthiae* from Japan.

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Material and Methods

Females of seven species of haplostomins from three localities, (1) various sta-

tions in the San Juan Archipelago, (2) Cape Flattery, Washington, and (3) the Whiffin Spit, Sooke, British Columbia, were available for the developmental studies. These species were collected from host ascidians obtained mostly sublittorally from the first locality by trawling and dredging, and intertidally from the last two areas.

The ovigerous females of the copepods were taken chiefly from spaces in the common test and rarely from zooids of the living ascidians. Individual females with egg sacs in pairs were maintained in 15 ml to 25 ml of filtered sea water in separate, embryologically clean, covered small petri dishes. In collecting the samples from the ascidians the egg sacs can easily be dislodged from the females. In such cases the egg masses in the sacs were reared without the female from the beginning. However, by preference, eggs which were ready for hatching and carried by the females were collected and cultured, because these could release nauplii naturally and successfully.

The approximate stage of development and the approach of the hatching time were indicated by the egg color. The low survival rate of eggs was mainly due to growth of ciliates and, probably, bacteria, and also the long culturing without the female. After the eggs were released from the egg sacs, the females were removed from the culture dishes. The water was changed regularly and the dishes cleaned daily or every two days. Each culture dish was put in another larger petri dish or glass container, which was also placed in a large flat aquarium.

The aquaria were supplied with circulating sea water from the glass-line system and the temperature of the water was usually 13°–14°C. The culturing was carried out mostly during the summer and autumn in 1966 and partly in the summer of 1965 and winter of 1967 at the Friday Harbor Laboratories of the University of Washington, Friday Harbor, Washington.

A certain number (usually 5 to 6 individuals) of the newly emerged first nauplii of representatives of each genus were isolated singly in culture dishes. By this method the periods of time of larval development from the napulii to the copepodids as well as the number of molts were determined. The times were recorded from what appeared to be typical individual examples successfully completing the developmental sequence from hatching to the copepodid stages. The times, thus, are not average or summarized figures but approximate actual examples. Because it was necessary to scrutinize several individuals during an observation period, it is possible for errors of one or more hours to apply to the time recorded for some stages. Many nauplii of the seven species were reared in mass culture in order to count the survival in the culture at each developmental stage or to obtain the larvae in later stages and additional larvae for the morphological study. Occasionally, some of the parasitic copepodids were directly collected from the host ascidians.

Morphological progression of the mouthparts of haplostomins was more distinctly distinguished in living larvae than in their exuviae. Therefore, anatomical details of the nauplii and copepodids, collected in various methods referred to above, were studied for the most part on the living larvae, some on fixed specimens in lactic

acid in which methyl blue was dissolved, and occassionally on exuviae. These larvae consist of various stages in each species in the descriptions.

Feeding for nauplii and early copepodids was not neccessary as all are lecithotrophic.

Results

I. Larval development of haplostomins

A. The genus *Haplosaccus*

Haplosaccus elongatus

The egg of *H. elongatus* is somewhat longer than wide, measuring about 0.18 mm in length. Approximately 300 ovate eggs are packed into a pair of elongate transparent sacs, which are hung by the anterior tips of the sacs on the spinose folds over the oviducal apertures of the female. The egg color is light purple, but before hatching a transparent pale yellow color is formed superficially, surrounding the original purplish color of the yolk in the middle. In such an egg (embryo) a large red eye is developed at the anterior end. When the eggs are ready to hatch, a longitudinal tear appears in the anterior part of each egg sac. At the same time the first nauplii come out from the opening of the sac, emerging from their egg membranes. The emerged nauplii swim briskly by moving the naupliar appendages. I did not study carefully the sensitivity of the embryos to stimulation by bright light, but it was noted that embryos would often hatch simultaneously soon after the culture dishes were illuminated under a binocular microscope.

The first naupliar molt of the individual selected for observation occurred about 12 hours and 30 minutes after hatching. The molt took place gradually, taking 1 minute from breaking of the exuvia until escape from it. The break occurred at the anteroventral part of the exuvia. After escaping from the exuvia the second nauplius swam actively. The third nauplius was obtained about 8 hours and 20 minutes after the first naupliar molt. About 11 hours and 20 minutes after the second naupliar molt, the third molt occurred, yielding the fourth nauplius. The fifth nauplius was obtained about 16 hours after the third naupliar molt. The free swimming first copepodid emerged about 17 hours after the fourth naupliar molt. Therefore, it took about 2 days, 17 hours and 10 minutes to reach the first copepodid after eclosion.

First nauplius: The large naupliar eye is reddish orange in color. It seems to be composed of two big dorsolateral ocelli and one ventral ocellus, which are almost colorless, and two central, reddish, pigment cells between the dorsolateral ocelli. A certain number of variably shaped pigment spots or concentrations, brownish in color, are distributed more or less symmetrically over almost all of the body: anterior and posterior to the naupliar eye; at the sides of the brain and midgut; and at the posterior end of the body. Around the midgut there are many round orange or yellowish globules, perhaps also containing yolk.

The body length is about 0.22 mm and the length relative to the width is about

1.7:1. The three pairs of naupliar appendages, antennules, antennae and mandibles, are supported on the ventral side of the upper one third of the body.

The uniramous antennule (Fig. 1 a-Al) consists of three articles, but articulation between the basal two (the first article is much shorter than the second) is not distinct: the basal article unarmed; the second atricle with 2 short setae; the distal article with 3 setae (1 long and 2 short).

The biramous antenna (Fig. 1 a-A2) consists of bimerous (articulation is indistinct) protopodite, unimerous endopodite and apparently trimerous exopodite. The basipodite has a lateral extension supporting the exopodite and this bears 1 seta at the mediodistal corner. This extension very likely represents the fused first article of the actually tetramerous exopodite. The endopodite bears 1 short medial seta and 2 long terminal setae. There are three complete articulations on the free expodite. The exopodite bears 5 setae: 1 long seta on each mediodistal corner of the basal two articles; 1 short medial, 1 long terminal and 1 short lateral seta on the third article. The third article gives by its outline and setation strong evidence that it actually is a complex representing two basic articles.

The mandible (Fig. 1 a-MD) has an indistinctly bimerous protopodite and endopodite, and tetramerous exopodite. The endopodite carries 1 short medial seta on the basal article and 1 short medial and 2 long terminal setae on the distal article. The armature of the exopodite consists of 4 long setae, each article with 1 seta. Posteriorly the larva has a pair of setae, showing the eventual positions of the caudal rami.

There is no rudiment of postoral appendages on the ventral surface in this stage. It is notable that there can be seen a certain number of specially enlarged cells, each including a large nucleus, arranged in order on the posterior half on the ventral surface. These cells seem to be related to a cell arrangement or distribution for making the thoracico-abdominal segments.

Second nauplius: The second nauplius (Fig. 1 b) has appendages similar to those of the first nauplius. However, behind these appendages three pairs of rudiments of mouthparts arranged in lateral series are developed under the cuticle. Each of the anterior two pairs of rudiments is a small hemispherical projection from the subcuticular body surface in ventral view. The remaining pair of rudiments is indistinct in shape but somewhat larger than the anterior.

Third nauplius: In each series of the "internal" rudiments of appendages the posteriormost rudiment is now distinguished as an enlarged rudiment of the maxilliped (Fig. 1 c). The two rudiments anterior to the maxilliped are seen as somewhat ovoid, and recognized as the rudiments of the maxillule and maxilla. The thoracico-abdominal area posterior to the maxillipeds covers distinctly less than half of the ventral surface. In this area the subcuticular first legs are seen in rudimentary form just behind the maxillipeds.

Fourth nauplius: The rudiments of maxillules and maxillae (Fig. 1 d) are internally seen as suboval pads. The maxillae lie behind the maxillules but rather anterolaterally to the large maxillipeds, which approach each other toward the midventral line. Each maxilliped has an apical pointed extension. In the thora-

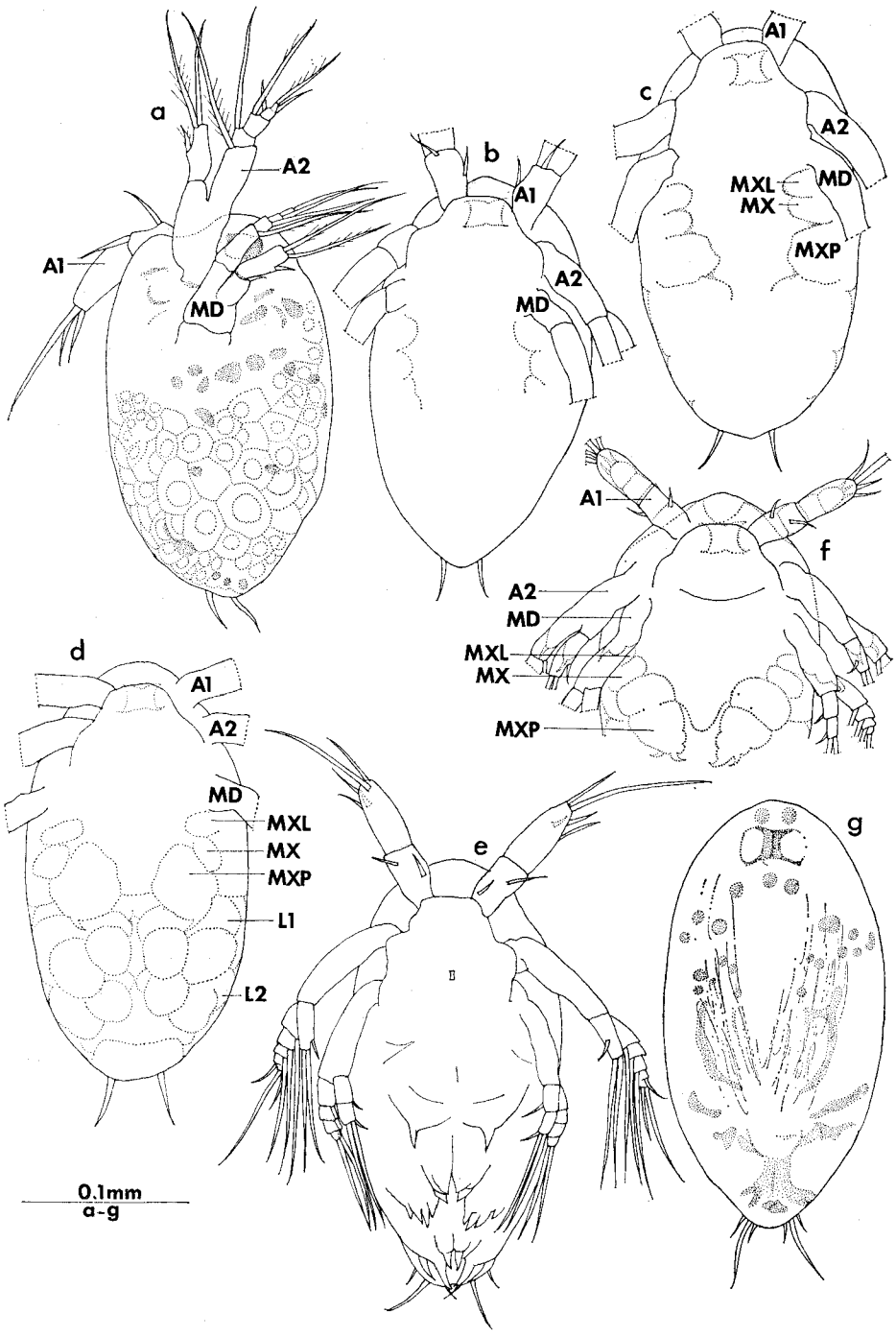

Fig. 1. Nauplii of *Haplosaccus elongatus* (a-d, f, g, based on living specimens): a, first nauplius (antennule, right; antenna, left; mandible, left), lateral; b, second nauplius, ventral; c, third nauplius, ventral; d, fourth nauplius, ventral; e, fifth nauplius (exuvia), ventral; f, fifth nauplius, anterior portion, ventral; g, fifth nauplius, showing pigment pattern, dorsal.

cico-abdominal area, the first and second legs are distinctly indicated, each consisting of unimerous protopodite and rami with a definite number of setae or spines in rudimentary form.

Fifth nauplius: The body length is about 0.24 mm, with the length relative to the width about 2:1. According to an exuvia (Fig. 1 e) of the fifth nuaplius the armature of the antennule is increased in number in comparison with that in the first nauplius, comprising 7 setae: the second article (without distinct articulation with the first article) with 2 short setae on the surface; the third article with 5 setae (4 short and 1 long) on the apical margin. The antenna and mandible seem comparable to those of the first nauplius in structure and armature.

Internally, however, features of the first copepodid (Fig. 1 f) are visible through the cuticle of the three pairs of naupliar appendages. The subcuticular uniramous antennule (Fig. 1 f-A1) consists of four articles, with a few setae at the apex. The subcuticular biramous antenna (Fig. 1 f-A2) is reduced in size and consists of the protopodite and the shortened endopodite and exopodite, all these apparently without any articulation. The endopodite is situated in the cuticle of the endopodite of the naupliar antenna whereas the exopodite reaches only to the outer extension (supporting the naupliar exopodite) of the naupliar protopodite. The subcuticular rami are rounded at the apices, but each has an apical pointed extension. The subcuticular mandible (Fig. 1 f-MD) is uniramous, situated in the protopodite of the naupliar cuticle. The apex is somewhat divided into two kinds of structures: inner rounded projection and outer tapering extension. The maxillule (Fig. 1 f-MXL) and maxillae (Fig. 1 f-MX) are situated internally behind the mandible, as in the preceding nauplius. The maxilliped (Fig. 1 f-MXP) tends to become a triarticulate structure including two large basal articles and a claw-like projection terminally; the distal point of the claw is posteriorly directed. There is a short pointed extension at the outer base of the claw.

In the exuvia of the fifth nauplius (Fig. 1 e) the maxilliped is seen as a simple conical sac with a posteriorly pointed extension, protruded from the exuvial surface without articulation. The first and second legs are seen as two pairs of lobes protruded in the same way. In each leg, the inner lobe representing the endopodite is much shorter and smaller than the other one for the exopodite. In the first leg the endopodite has 3 finger-like extensions posteriorly directed and the exopodite carries 4 similar extensions. In the second legs, similar extensions are developed but indistinct in number. The posterior end of the body has a medial terminal indentation, producing the terminal abdominal lobes which indicate the future caudal rami; each lobe with 1 terminal seta as well as 2 setules.

Before the molt for the first copepodid the larva becomes almost entirely transparent pale yellow (slightly orange in some specimens), but in the middle of the body the purplish color of yolk still remains. The brownish pigment spots can be seen more distinctly on the dorsal and ventral sides. In one specimen (Fig. 1 g) the arrangement of pigment spots on the dorsal side was observed as comprising two groups, the anterior and the posterior. The anterior group consists of more than

20 varied round pigment spots situated around the naupliar eye, brain and anterior part of the midgut. The posterior group is extended from the sides of the midgut toward the lateral margins of the body and also located on the proctodeaum, consisting of rod-shaped pigment spots varying from short to long. On the ventral side, several round or short rod-shaped pigment spots are discernible on the oral area and on the thoracico-abdominal area.

First copepodid: The body (Fig. 2 h, i) consists of cephalosome, metasome with two pairs of legs, and urosome. The length is about 0.372 mm, measured from the anterior tip of the cephalosome to the end of the caudal rami. The total length including the caudal setae is about 0.429 mm. The body color becomes overall transparent, slightly reddish orange. In the cephalosome and metasome there are many orange globules surrounding the big brownish midgut internally. In the cephalosome a certain number of variably shaped pigment spots is present behind the big red eye. In live specimens, this copepodid is easily distinguished from the swimming copepodid of *Haplostoma albicatum,* which will be described later, by the body color; the latter copepodid is transparent and slightly yellowish white in color.

The cephalosome is slightly longer than wide. The apex is projected ventrally into a well-developed rostrum, somewhat longer than wide in ventral view, with a subtriangular distal margin. The appendages (Fig. 2 j) consist of six pairs: antennule, antennae, mandibles, maxillules, maxillae and maxillipeds, as does the adult female, although their structure is not closely similar in the larva and adult. The midventral surface between the antennae and the mandibles is protruded ventrally into a conical lobe with a pointed apical extension. The mouth probably opens at the anterior base of the lobe.

The antennule (Fig. 2 j-A1) is tetramerous, bearing a few naked setae or setae and aesthetes on each article.

The antenna (Fig. 2 k-A2) is biramous, consisting of protopodite and rami, without definite articulation. The protopodite has no armature. The endopodite has 1 stout spiniform element at the apex, 1 similar but smaller element at the distal third on the medial margin and 1 vestigial element behind it. The exopodite is somewhat more slender than the endopodite, bearing 1 vestigial apical seta and without internal tissue within the cuticle. The antenna of the adult female consists of three articles armed with 2 spines (1 stout terminal and 1 smaller subterminal) on the distal article. The armature of the adult antenna basically corresponds to that of the endopodite of the copepodid. It is considered that the adult antenna is derived from the protopodite basally and endopodite distally.

As in the antenna the mandible (Fig. 2 k-MD) is biramous, consisting of the protopodite and rami, without definite articulation. The subcylindrical protopodite is unarmed. The short endopodite is stout and rounded at the apex, with 1 stout pointed extension. The exopodite is longer than the endopodite, but crumpled from distal to basal, with its cuticle withered distally. As the biramous mandible in the copepodid has the exopodite degenerating, it seems that the subcylindrical

uniramous mandible in the adult female is derived from the protopodite proximally and the endopodite distally, although the apex in the female mandible is slightly divided into two rounded lobules.

The maxillule (Fig. 2 k-MXL) is a small unsegmented lobe, somewhat longer than wide, with 1 short apical setule. It is protruded from the body surface behind the mandible and in front of the maxilla.

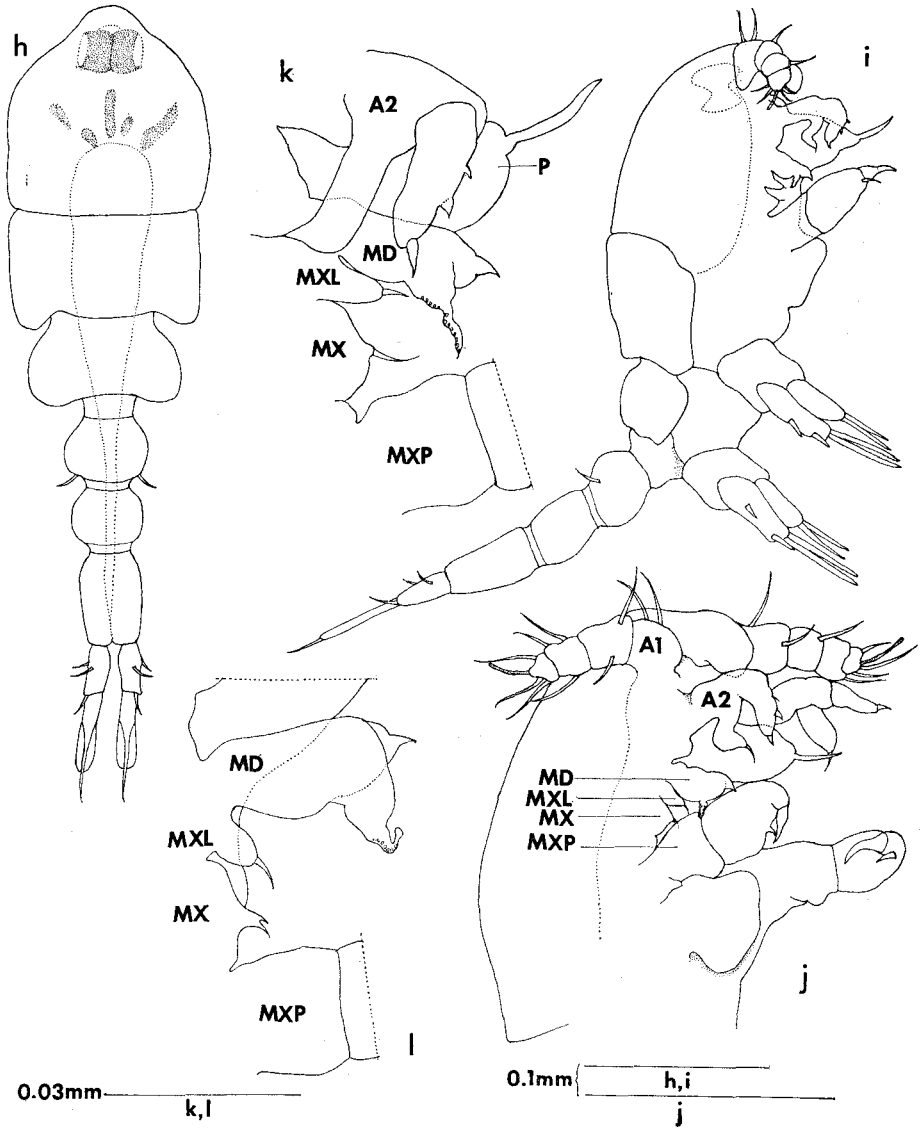

Fig. 2. First copepodids of *Haplosaccus elongatus* (based on living or fixed specimens): h, dorsal; i, same, lateral; j, appendages of cephalosome, lateral; k, oral area, lateral; l, mouthparts including maxilla with 2 setule-like extensions, lateral.

The maxilla (Fig. 2 k-MX) is represented as a subconical lobe, wider than long, situated anterolaterally to the maxilliped. The armature is the same as that of the maxillule. In one specimen (Fig. 2 l-MX), the apex of the appendage was divided into 2 setule-like extensions. In the adult female the maxillule and maxilla are each with a round lobe ornamented with several rows of spinules.

The maxilliped (Fig. 2 j-MXP) consists of two large basal articles with a terminal claw complex. The second article has a seta-like element on the mediodistal margin. The terminal claw complex includes an indistinct basal portion, which is seen as a separated third article in the adult female, and a slender terminal claw. In the female, the second article is without the seta-like element and the terminal claw has a spinule protruded from the medial margin near an articulative line at the proximal third.

The metasome (Fig. 2 h, i) consists of two segments. The first segment is as wide as the cephalosome and the second segment is much narrower than the first. Each segment has a dorsal plate-like sclerotization and a pair of swimming legs. Each leg consists of protopodite and rami. The segmentation and armature of these legs seem to be approximately comparable to those of the first copepodid of *Haplostoma albicautm* (see p. 267).

The narrow urosome (Fig. 2 h, i) consists of two segments and anal somite. The first segment is as large as the second segment, with a pair of seta-like indications for the third legs. The anal somite is the longest, bearing the small caudal rami. Each ramus (Fig. 2 h) carries 1 elongate terminal seta, which is about 2.5 times as long as the ramus. The seta is broad basally and tapers gradually. The proximal two thirds of the seta is fringed with broad hyaline flanges. The distal one third of the seta is naked. There are 3 small non-plumose setae besides the terminal seta: 1 at the distolateral corner, 1 at the proximal third on the lateral margin and 1 at the midpoint on the dorsal side but more laterally.

B. The Genus *Haplostoma*

Haplostoma albicatum

A pair of elongate cylindrical egg sacs, containing a white egg mass in each sac, is hung on the spinose oviducal folds of the female. Each egg measures about 0.18 mm in length. When the eggs (embryos) are near hatching they become somewhat transparent yellow in color, and acquire a brownish red or deep brownish naupliar eye in the anterior end of each ovate egg.

One hundred and three first nauplii were obtained from a pair of egg sacs of a female. This was the only case in which I made counts of the survival in the culture at each developmental stage. The percentage of survival for each stage until the first copepodid was calculated as follows:

Stage	Number of individuals	Percentage of survival
N1	103	100 %
N2	103	100 %
N3	77	75 %

N4	59	57 %
N5	37	36 %
C1	9	8.7 %

The approximate time periods elapsed in the five naupliar stages of the individual selected for observation were studied: 11 hours for the first nauplius; 12 hours for the second nauplius; 11 hours for the third nauplius; 18 hours for the fourth nauplius; 12 hours for the fifth nauplius. Therefore, it took about 2 days and 16 hours to reach the first copeopdid after eclosion. A verminform copepodid in a later stage was obtained from the host ascidian.

First nauplius: The body is transparent pale yellow in color and includes many globules and yolk masses surrounding the midgut which is not distinctive from the body color. Brownish red or deep brownish pigment spots are comparable to the naupliar eye in color and to those of *H. elongatus* in arrangement and in their varied shapes. The body length is about 0.22 mm and the relative length to the width is about 1.6:1, measured on the larva immediately after hatching.

The structure and situation of the three pairs of naupliar appendages and the caudal setae are fundamentally the same as those of *H. elongatus*. Behind the naupliar appendages there is no evidence of the mouthparts or legs, although on the dorsal side the body shows internally a tendency to divide into three divisions which are to be developed as the cephalosome, metasome and urosome in the first copepodid.

Second nauplius: The body length becomes more elongate, with the length relative to the width about 1.7:1. The naupliar appendages are about the same as in the first nauplii in structure and armature.

A pair of pad-like projections becomes internally visible as the rudiments of mouthparts, slightly protruded from the body surface behind the naupliar mandibles.

Third nauplius: The pad-like rudiments of mouthparts become more distinctive in appearance than in the second nauplius. At the same time the rudiments of maxillipeds, legs and caudal rami are developed as in the case of the third nauplius of *H. elongatus*. Each pad-like projection referred to above is situated at the anterolateral side of the rudiment of the maxilliped, with a slight space between the mandible and the projection. Considering the situation and shape, this projection probably corresponds to the rudiment of the maxilla which was observed in the second or third nauplii of *H. elongatus*.

Fourth nauplius: Within the cuticle of the naupliar appendages features of the first copeopdid are gradually formed.

Fifth nauplius: The body length becomes about 0.24 mm. The length relative to the width is about 2:1, as in the same nauplius of *H. elongatus*. The cuticle of the three pairs of naupliar appendages encloses structures of antennules, antennae and mandibles of the first copepodid. The thoracico-abdominal area behind the maxillipeds covers less than half of the ventral surface on the body as in the fifth naupliar stage of *H. elongatus*. However, the area of the body (Fig. 3 a) between the naupliar mandible and the maxilliped is not fully occupied by appendages,

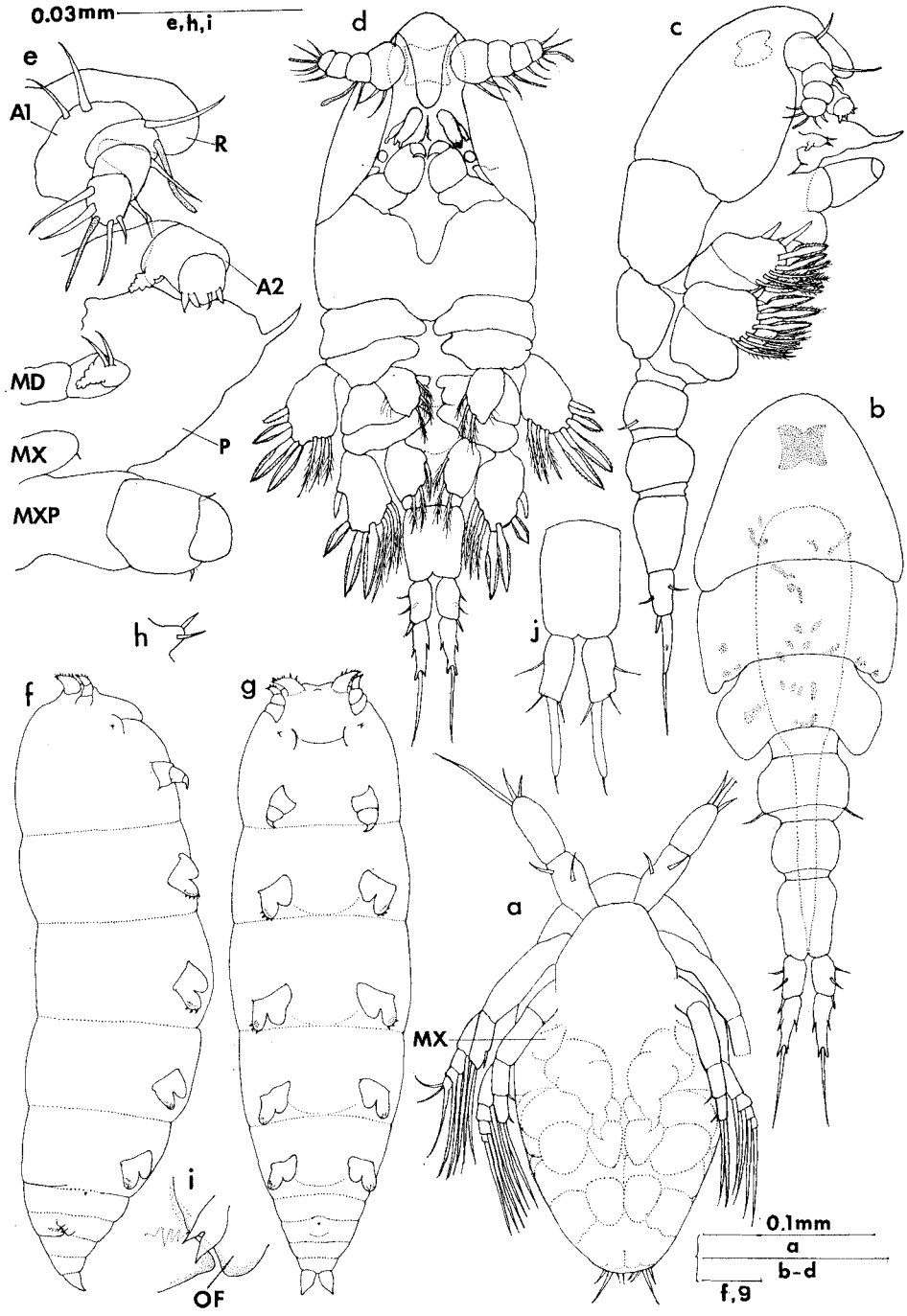

Fig. 3. Larvae of *Haplostoma albicatum* (a-i) and *H. elegans* (j): a, fifth nauplius, ventral; b, first copepodid, dorsal; c, same, lateral; d, same, ventral; e, same, appendages of cephalosome, lateral; f, copepodid female in a late stage, lateral; g, same, ventral; h, same, mandible (right); i, same, oviducal fold (right); j, first copepodid, caudal rami, dorsal.

unlike the corresponding area of *H. etongatus*; there is only a single rudiment of the maxilla visible. Two pairs of biramous legs with definite armature are distinguished within the cuticle. Features of the caudal setae resemble those of *H. elongatus*, consisting of a pair of setae and a few pairs of setules.

First copepodid: The body length is about 0.337 mm, measured from the tip of the cephalosome to the end of the caudal rami. The total length including the caudal setae is about 0.4 mm. The body structure (Fig. 3 b-d), with the well-developed rostrum with a subtriangular distal margin, a pointed conical projection from the midventral surface of the oral area, two pairs of swimming legs and a pair of caudal setae, is comparable to that of the first copepodid of *H. elongatus*. The cephalosome bears five pairs of appendages: antennules, antennae, mandibles, maxillae and maxillipeds. The coloration of the larval body, including eye, pigment spots, globules and midgut, which were observed in the first naupliar stage, remains the same, although the midgut tends to become more brownish in color in the copepodid.

The antennule (Fig. 3 e-A1) is composed of four articles ornamented with short non-plumose setae and aesthetes: the first article with 2 setae; the second article with 1 seta and 1 aesthete; the third article with 1 seta; the fourth article with 4 setae and 1 aesthete. The segmental composition is comparable with that of the adult male as well as all of the other males of *Haplostoma* species from North America, although in these males the basal article becomes an extremely expanded structure bearing a large number of characteristic aesthetes.

The antenna (Fig. 3 e-A2) is biramous. The protopodite consists of two articles, supporting unimerous rami. The endopodite bears 4 small pointed extensions, which are comparable to the armature of the adult (spines in the male and female). The exopodite is crumpled from distal to basal. Thus, it is apparent that the distal article of the trimerous adult antenna is derived from the endopodite, whereas the basal two articles are from the protopodite.

The mandible (Fig. 3 e-MD) is biramous as in the antenna but much smaller in size. The bimerous protopodite supports unimerous rami, without definite articulation. The endopodite is represented as a small lobe with 2 short apical non-plumose setae whereas the exopodite is crumpled from distal to basal. The mandible of the adult consists of an elongate (in the male) or subconical (in the female) lobe with 2 unequal (in the male) or subequal (in the female) setae. Therefore, it is obvious that the adult mandible is distally derived from the endopodite with similar armature in the copepodid.

The maxillule is absent. The maxilla (Fig. 3 e-MX) is situated anterolaterally to the maxilliped as a small lobe-like projection with 1 short apical setule. The structure and situation are basically comparable to those of the maxilla of the first copepodid of *H. elongatus* (see p. 284 for the further statement regarding the terminology of the maxilla).

The maxilliped (Fig. 3 d, e–MXP) is trimerous, consisting of two large basal articles and a small terminal article supporting a claw. The second article bears

2 setules anteriorly and posteriorly on the medial margin near the distal end. The structure and armature of the appendage are comparable to those of the adult maxilliped in both sexes.

The metasome is two-segmented. Each segment carries a pair of swimming legs (Fig. 3 d). Each leg consists of a bimerous protopodite and unimerous rami. The endopodite of the first leg is somewhat longer than wide, much smaller than the exopodite, and bears 3 medial plumose setae; the lateral margin is furnished with a few hairs and the apex is pointed. The exopodite is about 1.3 times as long as wide and ornamented with 3 subequal plumose setae, arranged on the medial margin, and 4 graduated spines, fringed with serrated hyaline flanges, on the lateral and distal margins. The spines gradually increase their lengths from basal to distal. In the second leg the armature of the rami is the same as that of the rami of the first leg.

The urosome consists of two segments and anal somite: the first segment with a pair of seta-like indications of the third legs; the second segment without armature; the anal somite with the small caudal rami terminally. Each caudal ramus (Fig. 3 b) has 1 characteristic long terminal structure, presumably a seta, 1 small spinule at the distolateral corner, 1 short naked seta at the distal third on the dorsal side, but more laterally, and 1 lateral similar seta at the proximal third. The terminal structure is about 3 times as long as the ramus and for its proximal half has the form of a thick elongate lobe, the distal margin of which is slightly divided into two lobules, and for the distal half a slender seta-like element which protrudes from the notch between the lobules. On each side of the thick structure there is a tiny projection from the margin.

A later parasitic copepodid: The body (Fig. 3 f, g) is expanded and gradually tapers posteriorly, producing a fusiform body appearance. The length is about 1 mm, measured from the anterior tip to the end of the caudal rami. It consists of the rather big cephalosome with four pairs of appendages, the obscurely four-segmented metasome with four pairs of thoracic legs, and the obscurely four-segmented urosome, not really set off by a major articulation, with rudimentary fifth legs on the first segment, rudimentary oviducal apparatus on the second segment and caudal rami without armature on the anal somite. The rostrum is not developed.

The cephalosome appendages on either side include an unsegmented conical antennule with short simple setae, a three-articulated antenna with 4 short spines on the distal article, a small subconical mandible (Fig. 3 h) with 2 short non-plumose setae and a three-articulated maxilliped. There is a big space between the mandible and the maxilliped as in the adult female. The maxilla which was observed in the first copepodid is not found in this copepodid. The mouthpart complement is the same as that of the adult female, differing from that of the first copepodid or the adult male.

The four pairs of thoracic legs resemble those of the adult female in structure and armature, although in the copepodid each exopodite has no lateral seta behind the usual lateral spines. The lateral spines, accompanied by many minute hairs

near their bases, in the first and second exopodites include 4 and 3 in number respectively as in the adult female. In the third and fourth exopodites of the copepodid the spines are not developed, but visible as rudiments through the cuticles.

The fifth leg is indicated by a tiny setule-like element situated on the first urosomal segment close to the distal end of the fourth metasomal segment. The segment of this leg is neither protruded laterally nor fused with the fourth metasomal segment anteriorly. In the adult female, however, the leg is represented as a lateral conical projection from the fourth metasomal section, bearing 3 setae which correspond to the setae of the fifth leg of the adult male.

The rudiment of the oviducal apparatus (Fig. 3 i) consists of a fold covering a slit-like aperture, situated on each dorsolateral side of the second urosomal segment. The fold bears 2 large strong spines, which are fused basally and protruded posteriorly from the surface of the fold, and 4 small spines arranged on the anteromedial margin of the fold and in front of the 2 main spines. The anterior spine of the 2 main elements is slightly smaller than the posterior one as in the adult female. In the adult female the small spine is articulated with the fold basally. The number of the main spines corresponds to that of the setae of the sixth leg of the adult male, indicating that the spinose oviducal fold is derived in part, at least, from the sixth thoracic leg.

The presence of the mouthparts, greatly reduced, and of the rudimentary fifth legs and oviducal apparatus indicates the sex of this copepodid. The copepodid can be designated as copepodid female and, presumably, by the following molt this copepodid female should result in the adult female.

Haplostoma elegans

Not each naupliar stage of *H. elegnas* was studied, but the first swimming copepodid was obtained about 5 days after eclosion in mass culture. A verminform copepodid specimen was also obtained about 29 days after eclosion in the same mass culture. By its appearance it was concluded that it represents the first parasitic stage capable of living in the host ascidian. However, the record of the culture does not furnish details to establish the stage appropriate for the copepodid with certainty.

First copepodid: The body structure is comparable to that of the first copepodid of *H. albicatum*. The mouthparts consist of mandibles, maxillae and maxillipeds. The mandible is a rather large subcylindrical lobe which is comparable to those of the male and female. The maxilla distinctly protrudes as a small lobe with 1 apical setule, and is situated anterolaterally to the maxilliped. In the male the appendage becomes a large subconical lobe with 1 short apical seta as in other males of *Haplostoma* species. In the female it remains as a small lobe with 1 or 2 short non-plumose setae.

The short caudal rami are like those of the first copepodid of *H. albicatum*, but their armature (Fig. 3 j) distinctly shows a tendency to the creeping form in this copepodid, each with a much shorter and characteristic terminal seta. The seta is about 1.6 times (3 times in *H. albicatum*) as long as the caudal ramus and for the most part (proximal four fifths) is covered by a thick elongated lobe-like structure

with smooth lateral and medial margins. The remaining part (distal one fifth) is naked, protruding from the distal margin of the thick lobe-like structure. There are 3 short simple setae additionally: 1 at the distolateral corner; 1 at the distomedial corner; 1 midway on the lateral margin. It is noted that the male of *H. elegans* belongs to the creeping type whereas the male of *H. albicatum* to the swimming form.

C. The Genus *Haplostomella*

Haplostomella distincta

The eggs of *H. distincta* were cultured from laying until hatching. A female was observed to extrude a pair of egg masses from her oviducal apertures in the laboratory. The period of development before hatching was 17 days. About 120 eggs were packed into the pair of elliptical egg sacs. The eggs when laid were reddish in color and about 0.15 mm in length (slightly longer than wide). After one week of culture the egg color gradually faded away from the superficial part. It is estimated that cleavage and gastrulation were completed by this time. After two weeks of culture, in each embryo a large red eye and four pairs of small round red pigment spots, which were arranged symmetrically, were developed. On the ventral side there is a single red pigment spot visible between the antennae. The egg masses remained attached to the female for 15 days and were viable after detachment until hatching. All of the eggs in the sacs hatched almost simultaneously as nauplii. However, these nauplii could not be cultured until further molting because of the growth of ciliate protozoans and, presumably, bacteria in the culture dish.

The approximate time periods elapsed for three naupliar stages of the individual selected for observation were as follows: 30 minutes for the first nauplius; 2 hours and 40 minutes for the second nauplius; 5 hours and 30 minutes for the third nauplius. The swimming first copepodid was obtained after the third nauplius. Therefore, it took about 8 hours and 40 minutes to reach the first copepodid after eclosion. The succeeding two copepodid stages (second and third) were collected from another series of culture. The second swimming copepodid was obtained about 14 hours after the molt for the first copepodid, and the third vermiform copepodid about 6 days after the molt for the second copepodid. The third copepodid appeared with a modified body configuration, approaching the appearance of the vermiform adult female, with the antennules, legs and caudal rami strongly modified toward the creeping form. Therefore, the second copepodid is the stage invading the host ascidian. Two kinds of specimens of the probable fourth copepodid stage were collected from the host ascidian. Copepodids in the subsequent stages of this species were not available in the samples. Probable fifth copepodids of the genus *Haplostomella* will be shown by the two species *H. oceanica* (p. 277) and *H. dubia* (p. 278) to disclose, in part, a sketch of the life history in the genus, because the full life history of haplostomins has never previously been presented.

First nauplius: The body length is about 0.168 mm, with the length relative to the width about 1.6:1. On the ventral side (Fig. 4 a) the upper one third is furnished with three pairs of naupliar appendages as in the *Haplosaccus* and *Haplostoma* species.

Internally the body shows a tendency to divide into five divisions (see Figure 4 b for the second nauplius). The first division is the largest, which is to be developed as the cephalosome of the first copepodid, occupying more than one third of the body length. The second to fourth divisions correspond to the first three leg-bearing segments of the first copepodid; these divisions gradually and posteriorly taper. The fifth division, posteriorly rounded, is to be developed as the urosome.

The four pairs of red pigment spots (see Figure 4 b for the second napulius) are arranged as follows: the first two pairs in the first division dorsally; the third pair in the third division dorsally; the fourth pair in the fifth division ventrally. It is noted that at the position of the single pigment spot between the antennae, the labrum will be developed in the later stages.

The ventral area posterior to the naupliar appendages has, internally, rudiments of mouthparts including probable maxillules and/or maxillae and also maxillipeds. These rudiments can be seen only with much dificulty because of their indistinct shape. The thoracico-abdominal area including the second to fifth divisions covers about half of the ventral surface; three pairs of legs are to be developed in the second to fourth divisions internally. There is a pair of caudal setae at the rounded terminal end of the larva.

Second nauplius: The second nauplius (Fig. 4 b) involves four pairs of small round pigment spots as seen in the first nauplius.

The naupliar antennule (Fig. 4 c-A1) has the internal tissue subdivided into four articles within the outer and inner cuticles; the inner cuticle corresponds to the third naupliar cuticle and the internal tissue shows the structure of the first copepodid.

A pair of pad-like rudiments of mouthparts are placed behind the naupliar mandibles, and are anterior, but somewhat lateral, to the rudiments of the maxillipeds. The position and shape of the pad-like rudiments (Fig. 4 c-MX) probably correspond to those of the rudimentary maxillae of the fourth or fifth nauplius of *H. albicatum*, although there is no space between the mandibles and the pad-like rudiments in this nauplius.

The first three divisions of the thoracico-abdominal area are each with a pair of rudiments of legs. Each leg bears a certain number of rudimentary setae or spines. The caudal setae are comparable to those of the first nauplius.

Third nauplius: The body length (Fig. 4 d, e) is about 0.176 mm, with the length relative to the width about 1.8:1.

The structure of the copepodid antennule (Fig. 4 e-A1) can be distinguished within the cuticle of the naupliar antennule. The reduced internal tissue of the antenna (Fig. 4 e-A2) is visible within the naupliar cuticle. The internal tissue of the mandible (Fig. 4 e-MD) is markedly reduced within the cuticle of the protopodite, forming a short lobe-like structure for the first copepodid mandible. The rudiment of the probable maxilla (Fig. 4 e-MX) becomes more distinct in appearance. The rudiment of maxilliped (Fig. 4 e-MXP) consists of two large articles; the second article includes a terminal claw-like element, but without articulation. The mediodistal corner of the second article is protruded into a rounded protrusion.

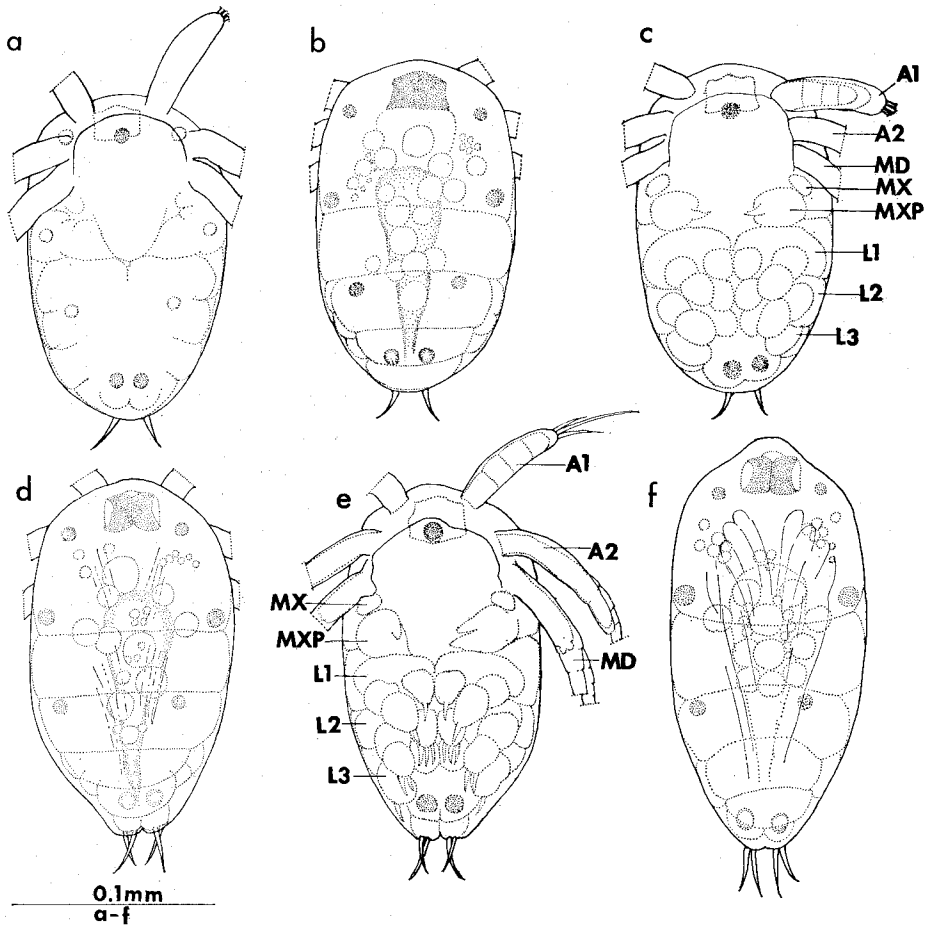

Fig. 4. Nauplii of *Haplostomella distincta* (based on living specimens): a, first nauplius, ventral; b, second nauplius, dorsal; c, same, ventral; d, third nauplius, dorsal; e, same, ventral; f, nauplius in a late stage, dorsal.

The thoracico-abdominal area covers more than half of the ventral surface of the body. In each rudiment of three pairs of legs (Fig. 4 e-L1～L3) in the area, the protopodite, endopodite and exopodite are distinguished. The caudal setae consist of two pairs.

It is noted that in one specimen (Fig. 4 f) obtained from a series of mass culture the body length was about 0.2 mm with the length relative to the width about 2:1. The body is elongate and somewhat fusiform. It is uncertain whether the nauplius belongs to the third or a later naupliar stage.

First copepodid: The first swimming copepodid (Fig. 5 g, h) is composed of cephalosome, metasome with three pairs of legs, and urosome. The body length is about 0.319 mm, from the tip of the cephalosome to the end of the caudal rami. The total length including the caudal setae is about 0.368 mm. The body encloses

a large red eye, slightly yellowish brain, and brownish midgut surrounded by many orange or yellow globules. The four pairs of round pigment spots are arranged at the sides of the eye, at the posterolateral corners of the cephalosome, at the lateral sides of the broad second metasomal segment and at the end of the anal somite. The rostrum is slightly divided at the apex into two lobes. The cephalosome bears five pairs of appendages: antennules, antennae, mandibles, maxillae and maxillipeds. It seems that there are two types of the first copepodid, one of them with biramous antennae, the other with uniramous antennae. In the specimen with the biramous antennae (Fig. 5 h) the exopodite is crumpled as in the case of *Haplosaccus* and *Haplostoma*. However, the cephalosome appendages of this specimen could not be observed in detail, so that the following description regarding the cephalosome appendages is based on the specimen (Fig. 5 i) with the uniramous antennae.

The antennule (Fig. 5 i-A1) is four-segmented, slightly tapering from base to apex, and ornamented with short non-plumose setae and aesthetes: the first article with 5 setae; the second article with 2 setae and 1 aesthete; the third article with 3 setae; the fourth article with 4 setae and 1 aesthete. The structure and setation are basically comparable to those of the first copepodid of the *Haplosaccus* and *Haplostoma* species. However, in the adult male the appendage is eight-segmented, with the armature including 5 extremely long aesthetes which are characteristic for the genus.

The antenna (Fig. 5 i-A2) is subcylindrical, without articulation. The armature includes 2 short medial setules: 1 at the distal third on the margin; and 1 near the tiny mammiform projection from the apex. The antenna is basically comparable to that of the adult male and female in appearance.

The mandible (Fig. 5 i-MD) is a short but rather large unimerous lobe, situated just behind the antenna and before, but more medial to, the maxilla. The apex has three small projections, two of which are rounded at the apices and the remaining innermost projection is pointed.

The maxilla (Fig. 5 i-MX) is a subconical lobe with 1 short apical setule, situated anteriorly to the maxilliped. In comparison, the lobe is more developed in this copepodid than in the *Haplostoma* species.

The maxilliped (Fig. 5 i-MXP) is a three-articulated structure, resembling the more complex appendage in the male and female. The mediodistal corner of the second article is distinctly protruded into a rounded projection, bearing 1 spiniform element on the anteromedial side near the distal end. The third article is claw-like, with the tip curved medially.

The metasome (Fig. 5 g, h) is three-segmented, each segment with a dorsal plate-like sclerotization and a pair of swimming legs. The structure and armature of the legs seem to be little different from those of the second copepodid. The third segment is much smaller than the first two segments.

The narrow urosome (Fig. 5. g) consists of two segments and anal somite. The anal somite bears a pair of caudal rami. Each caudal ramus carries 2 long terminal setae (the medial seta is somewhat longer than the lateral one) fringed with hyaline

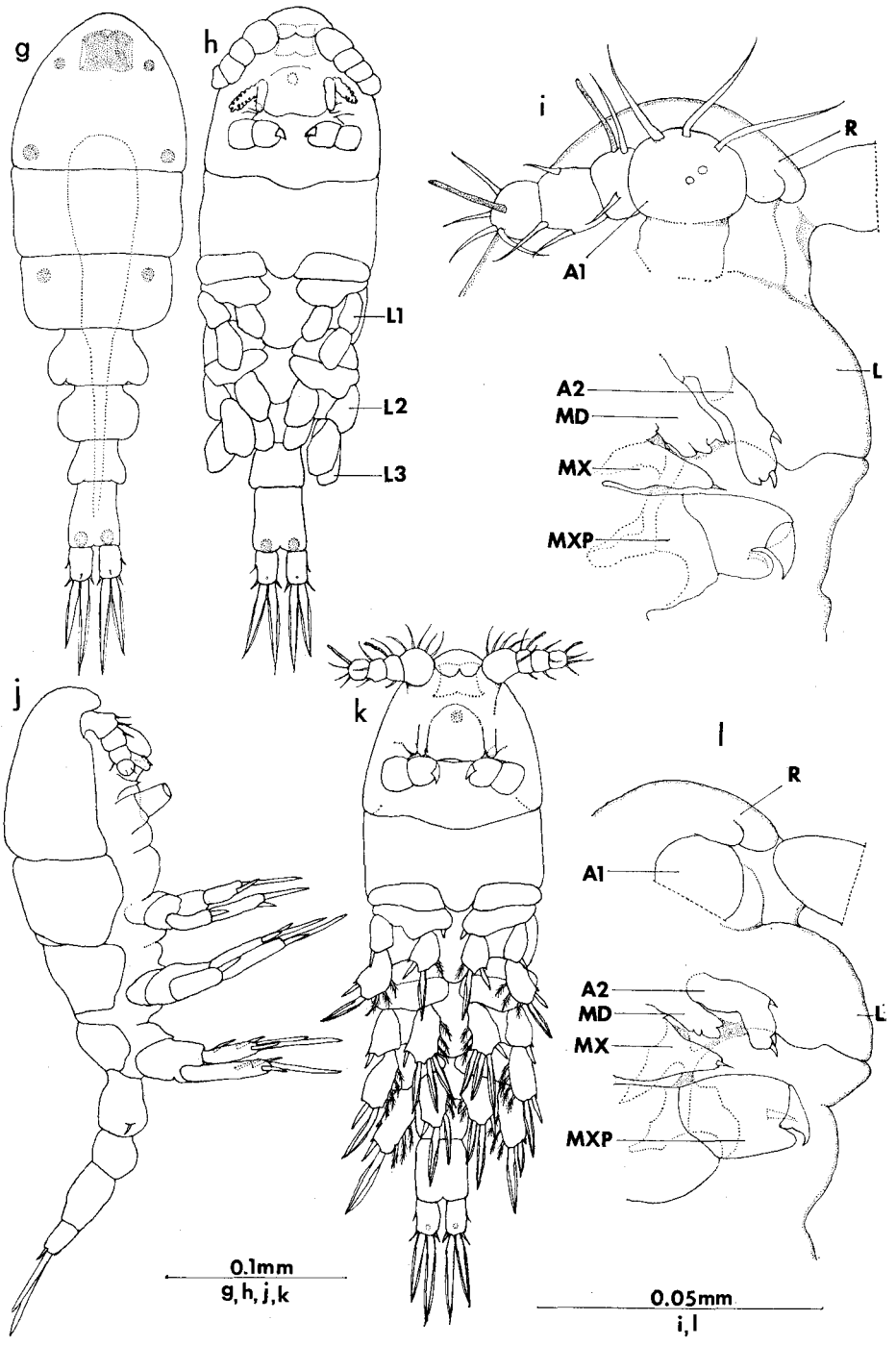

Fig. 5. Copepodids of *Haplostomella distincta* (based on living or fixed specimens): g, first copepodid, dorsal; h, same, ventral; i, same (based on different specimen), appendages of cephalosome, lateral; j, second copepodid, lateral; k, same, ventral; l, same, appendages of cephalosome.

flanges, 1 spinule at the mediodistal corner, 1 similar spinule at the distolateral corner, 1 dorsal setule near the distal margin and 1 lateral setule midway on the lateral margin. The arrangement of the armature is comparable with that in the adult male.

Second copepodid: The body length and structure (Fig. 5 j, k) closely resemble those of the first copepodid. The first urosomal segment bears a seta-like indication for the fourth leg on either side.

The antennule (Fig. 5 k) is almost the same as that of the first copepodid. The antenna (Fig. 5 l-A2) has 1 apical setule added to the armature, so that it has 3 setules. The mandible (Fig. 5 l-MD) is somewhat reduced in size, but still remains as the lobe-like structure with three small projections apically. Maxillule is absent. The maxilla (Fig. 5 l-MX) is a conical lobe with an apical seta as in the first copepodid. The maxilliped (Fig. 5 l-MXP) is comparable to that of the first copepodid.

The first leg (Fig. 5 k) consists of bimerous protopodite, unimerous endopodite and bimerous exopodite. The basipodite has 1 short spine at the mediodistal corner. The endopodite bears 1 short medial plumose seta and 2 spines around the apical margin. The exopodite has 1 small lateral spine on the first article and 3 medial plumose setae and 2 apical spines on the second article. The second leg is comparable to the first leg in structure, but differing in armature. The endopodite bears 3 medial setae and 4 spines on the apical margin. The exopodite has 1 lateral spine on the first article and 4 medial setae and 2 apical spines on the second article. The third leg consists of bimerous(?) protopodite and unimerous rami: the endopodite with 2 medial setae and 3 spines on the apical margin; the exopodite with 3 medial setae, 2 apical spines and 1 lateral spinule.

Third copepodid: The vermiform body (Fig. 6 m-o) gradually tapers posteriorly and has a slight dorsal curvature. The length is about 0.348 mm, from the anteriormost to the end of caudal rami. The body is divided into a cephalosome with five pairs of appendages, a metasome of three leg-bearing segments, and a three-segmented urosome, the first with rudiments of the fourth legs and the last (anal somite) with small caudal rami. The rostrum is not developed. The body is transparent and yellowish white in color, marked by a big red eye, yellowish brain, big brownish midgut and a certain number of pale greenish globules. The four pairs of red pigment spots are situated as follows: the first pair in the cephalosome; the second and third pairs in the first and second metasomal segments, and the fourth pair in the anal somite.

The antennule (Fig. 6 n) is an unsegmented conical lobe with a small number of short non-plumose setae on the margin. The antenna (Fig. 6 p-A2) is basically comparable to that of the swimming copepodids in structure, but the armature is more developed in this copepodid with 4 setules.

The mandible (Fig. 6 p-MD) is a spiniform projection on a slightly expanded base, accompanied by a small rounded projection posterolaterally, and is situated behind the antenna and close to the lateral extent of the mouth-opening. The spiniform mandible would probably be formed through the growth of the innermost

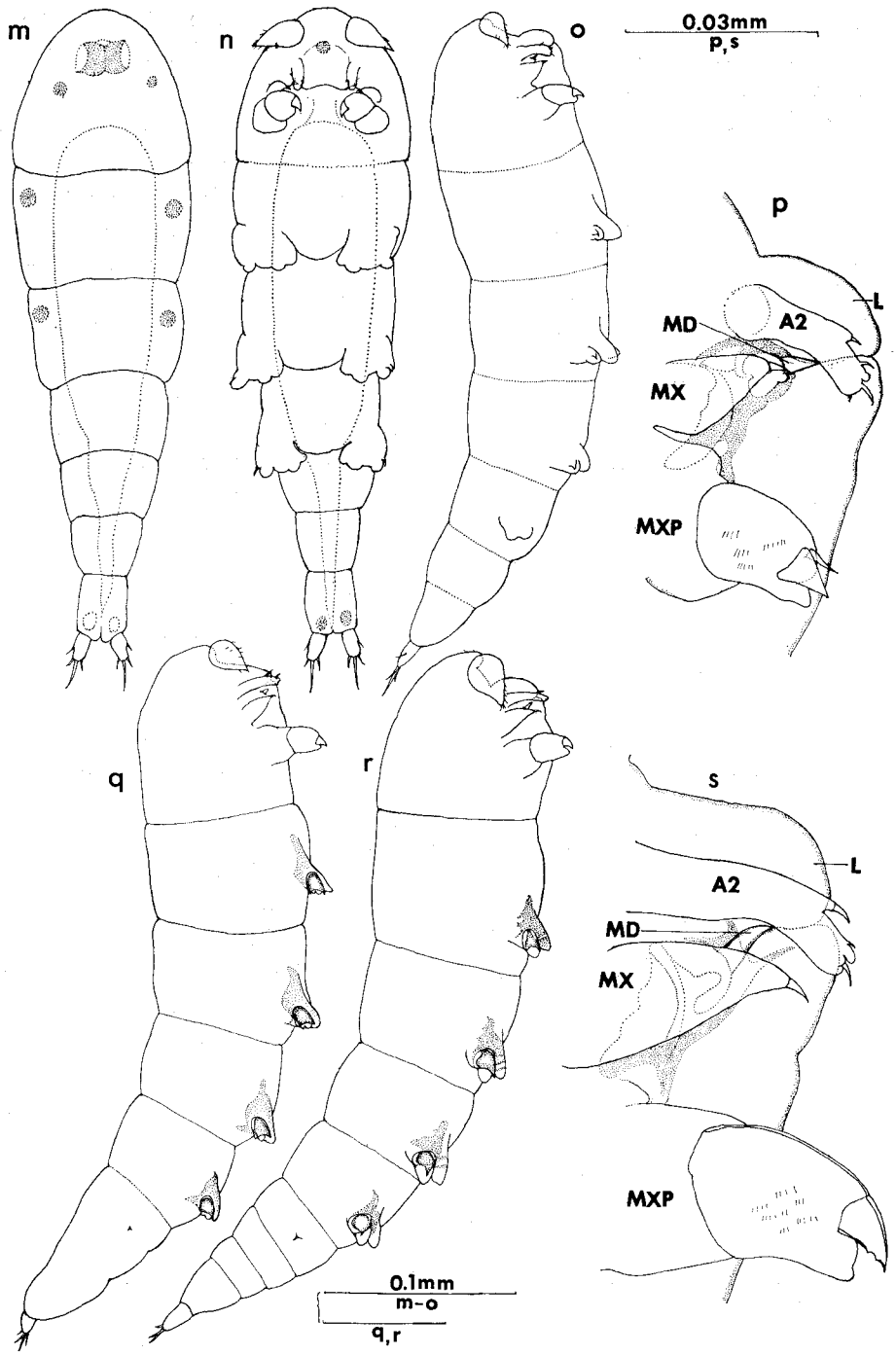

Fig. 6. Copepodids of *Haplostomella distincta* (based on living or fixed specimens): m, third copepodid, dorsal; n, same, ventral; o, same, lateral; p, same, oral area, lateral; q, probable fourth copepodid with unsegmented urosome, lateral; r, probable fourth copepodid with segmented urosome, lateral; s, same, oral area, lateral.

projection of the three apical projections of the lobe-like mandible in the swimming copepodids. It seems that the greater part of the lobe-like mandible is shortened in the meantime and one of the remaining rounded projections remains at the posterolateral base of the spiniform projection. The spiniform mandible with such an accessory element is comparable to that of the adult male. In the adult female the mandible consists of a single spiniform projection without any accessory element.

The maxilla (Fig. 6 p-MX) is a distinct conical lobe, bearing 1 apical setule as in the swimming copepodids. The three-articulated maxilliped (Fig. 6 p-MXP) is provided with rows of denticles on the second article.

The first to third legs (Fig. 6 n, o) resemble each other and approach the configuration of those in the adult female. Each leg is protruded from the body surface into a broad unsegmented structure bilobed apically. The basal part probably includes the protopodite whereas the apical lobes comprise the endopodite and exopodite. The endopodite is slightly divided into two similar rounded lobules at the apex. The exopodite is smaller than the endopodite and somewhat more differentiated in structure, because a small pointed projection is protruded from the anterolateral surface of the finger-shaped ramus.

The rudiment of the fourth leg (Fig. 6 o) is represented only as a small lobe situated rather laterally on the first urosomal segment. The apex of the rudiment is slightly protruded from the segment into two small rounded projections representing the endopodite and exopodite.

The caudal ramus (Fig. 6 m, n) is twice as long as wide, bearing 4 short nonplumose setae: 1 seta terminally; 2 setae at the sides of the terminal seta; 1 lateral seta at the middle on the margin.

Fourth copepodid: In one specimen (Fig. 6 q) of the probable fourth copepodids the body length including caudal rami is about 0.6 mm. The general configuration corresponds to that of the third copepodid, with the cephalosome relatively large and the posterior body vermiform. The complement of mouthparts is comparable to that of the third copepodid. However, the mandible consists of a single spiniform element without an accessory element as in the adult female. The fourth legs occupy the usual position for the thoracic legs, lying behind the third legs. Therefore, the metasome consists of four leg-bearing segments and becomes elongated. Through the four pairs of legs the anterolateral pointed structure of each exopodite becomes sclerotized and the ramus is articulated basally. The legs show a strong similarity to those of the adult female. However, there are neither dorsolateral plates nor lateral tubercles associated with the thoracic legs. The urosome, indistinctly delimited from the metasome, is unsegmented. There is a tiny setule-like indication of the fifth leg at the proximal fifth on each lateral side of the urosome. The situation and appearance of this structure show a correspondence to the fifth leg in the adult female, although in the female the leg is represented by 2 unequal setules.

In the other specimen (0.66 mm in length) the urosome has a four-segmented structure, the first segment with the tiny rudimentary fifth legs (Fig. 6 r). The

mouthparts (Fig. 6 s) are the same as those of the former specimen. Four pairs of thoracic legs are basically comparable to those of the former specimen, but the sclerotization of the endopodites and exopodites shows a tendency to division into two or three articles.

Haplostomella oceanica

Some 80 ovate eggs are packed into a pair of elongate transparent ovisacs. The eggs (embryos) are purplish in color in the early embryonic stages. The larval development from the first nauplius to first copepodid is comparable to that of *H. distincta*, with an abbreviation in the naupliar stages. Although the time period elapsed for each naupliar stage was not studied, the first naupliar molt occurred about 30 minutes after eclosion as in *H. distincta* and the first copepodid was obtained about 13 hours (perhaps including the time periods for the second and third naupliar stages) after the first molt. The pigmentation pattern (purplish red in color) in these larvae is also comparable to that in *H. distincta*. A probable fifth copepodid was obtained from the branchial sac of the host ascidian.

Fifth copepodid: In the presumed fifth copepodid (Fig. 7 a) the body (0.85 mm in length) is provided with many of the features similar to those of the adult female, with the cephalosome relatively small, the elongated metasome bearing four pairs of thoracic legs of creeping form. However, the metasome is still four-segmented (five-segmented in the adult female) and the segment for the fifth legs is included in the relatively elongate urosome. The urosome is unsegmented, indistinctly articulated with the metasome, and bears two pairs of tiny appendages situated at the positions for the fifth legs and the oviducal apertures of the female. Therminally, there is a pair of short caudal rami.

As in the adult female the armature of the antennule (Fig. 7 b-A1) includes three kinds of naked setae; 1 stout seta at the apex, 1 longer seta at the anterior base of the apical one and directed proximally, and 1 tiny seta between them. The antenna (A2), mandible (MD), maxilla (MX) and maxilliped (MXP) are comparable to those of the adult female or male in structure and armature, although the maxilla has 2 relatively long apical setae; in the adult the setae of the maxilla are not so long in either sex.

In the metasome (Fig. 7 a), the four pairs of legs are more developed than in the probable fourth copepodid of *H. distincta*, bearing 1 apical setule on the unsclerotized lobule of each exopodite.

In the urosome the anterior small appendage (Fig. 7 a) is represented by 2 setules placed on a slightly expanded base, situated laterally at about the proximal fourth. The leg corresponds to the fifth leg of the adult male and female in structure, armature and general situation. The more posterior appendage placed at about the distal third on each lateral side of the urosome consists of 2 tiny medial spinules and 1 longer lateral setule, arranged side by side on the distal margin of a small semicircular lobe-like structure protruded from the urosome. The structure of the appendage can be compared with the sixth leg of the adult male as well as other *Haplostomella* males, in which 2 small medial spinules and 1 long lateral seta are

placed on the distal margin of the lappet-like lobe from the second urosomal segment.

The location of the sixth leg of the present copepodid also corresponds to the position of the oviducal aperture of the adult female. In the adult female the oviducal aperture is covered by an oval plate with a free distal margin, which forms a sclerotized fold. The undersurface of the fold has a transverse row of 3 subequal small spines protruded from it. In all the females of the North American species of *Haplostomella,* the oviducal fold is of similar structure, always including 3 spines. The supposition here is that the fold is derived from the simply structured sixth appendage of the copepodid, by turning in the distal margin of the appendage from outside to inside. At the same time the oviducal aperture probably opens under the appendage and the adjacent body surface comes to be sclerotized, forming the oviducal plate over the aperture. The formation of the fifth legs and oviducal apparatus in the adult female will probably be achieved after passing through the following molt in this female copepodid.

Haplostomella dubia

The first naupliar larvae of *H. dubia* are notable in the lack of a naupliar eye (naturally the larvae in later stages and the adult lack the eye), but in the usual position of the eye a small pale pigment mass is found. Four pairs of round pigment spots arranged at the sides of the body and a single pigment spot located between the antennae are green in color. Moreover, there is a single red pigment spot behind the green spot between the antennae. At the position of the red spot the labium will be developed in later stages.

Thirty-seven first nauplii from a single ovisc were obtained about 30 minutes after eclosion (a pair of elliptical oviscs usually contains 60–70 eggs in number). From these nauplii 9 first swimming copepodids were collected in mass culture, taking about 7 hours and 50 minutes after eclosion (the periods for the second and third naupliar stages were not available for examination). Seven of the 9 first copepodids reached the second swimming copepodid stage, taking about 13 hours after the molt for the first copepodid. A few specimens of the probable fifth copepodid stage were collected from the branchial sacs of zooids of the host ascidian.

First and second copepodids: The following description is restricted to the mouthparts of the first and second copepodids.

In *H. dubia*, which has a lobe-like mandible in the adult male and female, the morphogenesis of the mandible is somewhat different from that of *H. distincta* and *H. oceanica* where the mandible is spiniform in the adult. In the first copepodid (Fig. 7 c) of *H. dubia* the cuticle of both of the endopodite and exopodite tends to crumple and a small unimerous cylindrical structure (Fig. 7 d-MD) can be seen at the base (protopodite) of the appendage internally. In the second copepodid (Fig. 7 e), the cylindrical structure (Fig. 7 f-MD) is clearly demonstrated, carrying an apical seta. This mandible is basically comparable to that of the adult male and female, although in the female it is obscurely and partially two-segmented. The maxilla (Fig. 7 f-MX) and maxilliped (Fig. 7 f-MXP) of the copepodid are developed as seen in *H. distincta*.

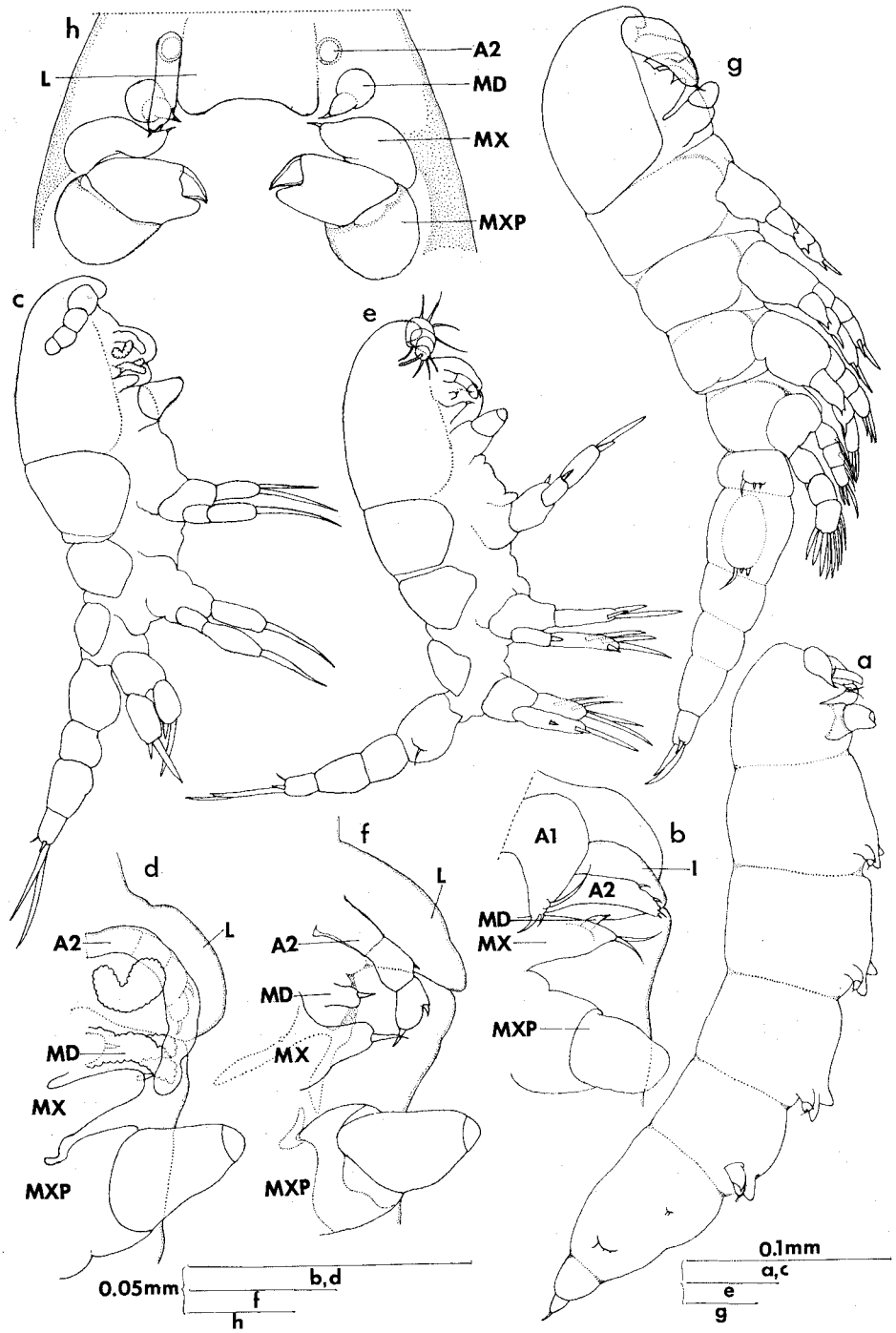

Fig. 7. Copepodids of *Haplostomella oceanica* (a, b) and *H. dubia* (c-h): a, copepodid female, lateral; b, same, appendages of cephalosome, lateral; c, first copepodid, lateral; d, same, oral area, lateral; e, second copepodid, lateral; f, same, oral area, lateral; g, copepodid male, lateral; h, same, oral area, ventral.

Fifth copepodid: The probable fifth copepodid (0.87 mm in length) shows many features of the male, including the big cephalosome with five pairs of appendages, the four-segmented metasome with four pairs of legs (each leg consisting of bimerous protopodite and trimerous rami), and the apparently five-segmented urosome (six-segmented in the adult male) with fifth and sixth legs, and with the second segment enclosing developing spermatophores (Fig. 7 g).

The mouthparts (Fig. 7 h) are comparable to those of the adult male, consisting of mandibles, maxillae and maxillipeds. The fifth leg (Fig. 7 g) consists of 2 small medial setules and 1 naked lateral seta arranged on the distal margin of the slightly protruded small lobe on the first urosomal segment. The sixth leg (Fig. 7 g) situated on the second urosomal segment is represented by a small lobe bearing 2 medial spinules and 1 lateral naked seta. The innermost spinule is slightly longer than the remaining spinule; in the adult male the longer spinule becomes a stout spine characteristic for the species.

Sexual dimorphism is evident in this copepodid with the above-mentioned features. However, this male copepodid is the creeping type, with short setae on the unsegmented antennule (eight-segmented in the adult male), with relatively short spines and setae on the thoracic legs from the first to fourth, and with short caudal rami with short setae. It is noted that one of the male copepodids could swim after molting in the petri dish in which the specimen was temporally cultured. The swimming-form male could conceivably be referred to as the sixth copepodid or adult male.

Haplostomella reducta

The larval development of *H. reducta* is basically comparable to that of *H. distincta*. The pigmentation pattern (red in color) in these larvae is also the same as that of *H. distincta*. The period for each larval stage was not studied, but the first naupliar molt occurred between 5 and 60 minutes after eclosion. It took about 12 hours to reach the swimming first copepodid stage and about 22 days until the vermiform third copepodid stage after hatching.

The reduction of the internal tissue of the mandible is remarkable at the second naupliar stage. It is much greater in the right mandible than in the left one. The internal tissue of the right mandible is visible through the naupliar cuticle only as a tiny rounded projection from the body. The conjecture here is that because the appendage is generally indistinct in both sexes in the adult, further reduction of the mandible will take place through the later naupliar and early copepodid stages. The vermiform copepodid is comparable to the parasitic third copepodid of *H. distincta* in general structure. It is noted that the first nauplius had the length of about 0.19 mm and the third copepodid reached about 0.47 mm in *H. reducta*.

II. Types of larval development of haplostomins

From the periods of time involved in the larval stages from first nauplius to first copepodid of seven species of haplostomins and the total number of their molts in these periods, it was found possible to divide the pattern of development of this

small subfamily into two types (Table 1).

The first type (Type I) is found in *Haplosaccus* and *Haplostoma* species, in which the period from hatching to first naupliar molt is more than 10 hours (about 12 hours in *Haplosaccus* and 11 hours in *Haplostoma*) and the period from hatching to first copepodid is 2 or more days (about 2 days in *Haplosaccus* and 2–5 days in *Haplostoma*) and requires a passage through at least five naupliar molts.

In the second type (Type II), which is found in the *Haplostomella* species, these periods are remarkably shortened, with the period from hatching to first naupliar molt generally taking less than 1 hour (about 30 minutes in *H. distincta*, *H. oceanica* and *H. dubia* and 5–60 minutes in *H. reducta*) and the period from hatching to first copepodid taking less than 1 day (7–9 hours in *H. dubia* and *H. distincta* or 12–14 hours in *H. reducta* and *H. oceanica*), and requiring at least three naupliar molts.

Table 1. Two types of the larval development of haplostomins, characterized by the periods for larval stages and by some morphological features of naupliar larvae and first copepodids. As, anal segment; C, cephalosome; Cl, first copepodid; CR, caudal ramus; L1-L3, legs 1–3; M1-M3, metasomal segments 1–3; NL, naupliar larvae; NM1, first naupliar molt; U1-U2, urosomal segments 1–2; TS, terminal setae.

Type	I		II
Specimen examined	*Haplosaccus*	*Haplostoma*	*Haplostomella*
	H. elengatus	*H. albicatum*	*H. distincta, H. oceanica H. dubia, H. reducta*
Period from hatching to NM1	more than 10 hours		less than 1 hour
Period from hatching to Cl	more than 2 days		less than 1 day
Pigmentation pattern of NL & Cl	variably shaped		definitely shaped
Segmentation & main appendages of Cl	C M1 ..... L1 M2 ..... L2 U1 U2 AS CR ..... 1 TS		C M1 ..... L1 M2 ..... L2 M3 ..... L3 U1 U2 AS CR ..... 2 TS
Genera to be included	*Haplosaccus, Haplostoma Haplostomides*		*Haplostomella*

These two types of development are correlated with morphological features of the embryos or larvae. In the embryos with the first type of development, pigment spots which appear in the embryos before hatching and can be observed even in the late larval stages are variably shaped, with mostly brownish and reddish or deep brownish color. Their last nauplii bear a pair of caudal setae. In the first copepodids the body is characterized by a long midventral projection from the oral area in the cephalosome, two pairs of swimming legs in the metasome and a single pair of terminal caudal setae.

The second type of development comprises the following morphological features in the corresponding embryos or larvae: the pigment spots are definitely shaped,

basically consisting of four pairs of red, purplish red or green round pigment spots; the last nauplius has two pairs of caudal setae; the first copepodid, without the ventral projection from the oral area, has three pairs of swimming legs and two pairs of terminal caudal setae. The segmental composition of the tagmata of the first copepodid is different from that of Type I (Table 1).

Applying these facts, the developmental type for the remaining genus *Haplostomides*, for which I did not determine most of the developmental stages because of the unsuccessful culture of the larvae after the first nauplius, can be estimated by the pigmentation pattern in its embryos or first nauplius. The eggs of *H. luteolus* are generally transparent yellow, rarely pale purple, in color. The embryos before hatching and also the first nnuplii acquire a big red eye, slightly orange gut, many yellowish globules and variably shaped brownish pigment spots. Considering just the characteristic of the pigment spots, the development of this genus seems to be included in the first type (Type I) and should be accompanied by the above-mentioned morphological features of the last nauplius and first copepodid (Table 1).

The division of the larval development into two types corresponds to that of dividing the subfamily into two series by morphological features (in first to fifth throacic legs and oviducal apertures) of females in interpreting the phylogenies (see Ooishi & Illg, 1977, pp. 145–157).

III. The rationale for the terminology of the mouthparts of haplostomins.

Through all of the species referred to above, the morphogenesis from the biramous mandible to the uniramous mandible takes place basically in the same way, accompanied by the degeneration of the exopodite (*Haplosaccus elongatus*; *Haplostoma albicatum*) or perhaps both of the endopodite and exopodite (*Haplostomella distincta*; *H. oceanica*; *H. dubia*). In these species the mandible is reduced in the first copepodid stage but never disappears. This fact is an important proof necessary to understand the terminology used for the mouthparts of the North American species. In addition to this fact, it is also very possible that in some haplostomin species the mandible reduction in the larval stages would take place to a greater degree, so that the appendage becomes indistinct as seen in *Haplostomella reducta*.

Regarding the constitution of the mouthparts, there are distinct relations between the larvae and the adults within each genus or species from North America. In other words, if the adult males or females lack certain mouthparts their larvae also show the same or similar condition. The developmental origin of the mouthparts except for the mandible could be ascertained by recognition of these larval homologies of mouthparts in the adults.

In *Haplosaccus elongatus*, two rudiments of mouthparts appear behind the mandible and before the maxillipeds in the naupliar stages. In the first copepodid these rudiments become two small subconical lobes with armature and can be designated as the maxillule and maxilla. The same arrangement of the mouthpart is found in the adult female.

In the corresponding larval stages of *Haplostoma albicatum* and *Haplostomella*

distincta, the mouthpart area between the mandible and the maxilliped is occupied by a single mouthpart. The single mouthpart is observed to be situated in the position for the maxilla of *Haplosaccus elongatus.* Therefore, it is concluded that the missing mouthpart in the larval stages is the maxillule. The same condition, the absence of maxillule, remains in the adult males of all of the *Haplostoma* species, the female of *H. elegans* and all of the males and females of the *Haplostomella* species.

However, in their naupliar stages the rudimentary maxilla of the *Haplostoma* and *Haplostomella* species appears as a pad-like structure which closely resembles both the rudimentary maxillule and maxilla in the naupliar stages of the *Haplosaccus* species. In view of this fact, it is rather difficult to distinguish the rudimentary maxilla at its first appearance in the former species. Therefore, the above-mentioned conclusion may not be based on a strong rationale.

Since the present conclusion may require further substantiation, additional statements or proofs can be assembled. For this purpose, a comparison of the mouthparts in the adult males of four genera, *Haplosaccus, Haplostomides, Haplostoma* and *Haplostomella,* would be useful, the reason being that the appendages corresponding to the missing mouthaprts in the latter two genera probably would be present in the males of the former two genera which are known to have the full set of mouthparts in their females.

In general, the collecting of the males of haplostomins is somewhat difficult as compared with the case of the females. Therefore, there was no description of males on record before our taxonomic studies on the North American species (Ooishi & Illg, 1977). Our collecting for males was unsuccessful in *Haplosaccus* and *Haplostomides.* However, there is a very useful male, unfortunately without positive identification, available for comparison.

The male in question (Fig. 8 a-g) is provided with mandibles, maxillules, maxillae, maxillipeds, having many features in common with the males of *Haplostoma* in body structure, antennules, antennae, thoracic legs and caudal setae. The host ascidian of this male is unknown because of a mistake in recording. Although there are several possibilities as to the generic identification, it is most probably a species of *Haplostomides* for the following reasons: (1) the males of *Haplostomides* must closely resemble those of *Haplostoma* in general structure, because the females of both the genera are very comparable to each other; (2) the most definite feature to distinguish these females is their mouthpart complement, so the males of *Haplostomides* also should probably be distinguished from the males of *Haplostoma* in their mouthparts.

In detailed comparison, the mouthparts of this male and all of the *Haplostoma* males exactly correspond to each other in arrangement, basic structure and armature, except that the maxillules are absent in the *Haplostoma* males (Ooishi & Illg, 1977, p. 31, Fig. 3). The maxillule of the present male is extremely small in size, but distinctly situated in a narrow space just behind the mandible (Fig. 8 b, c, e). A comparable but somewhat narrower space is found in the mouthpart area of the males of *Haplosoma,* without the appendage. These facts strongly support the conclusion that in the males of *Haplostoma* the maxillules are absent and the mouthparts

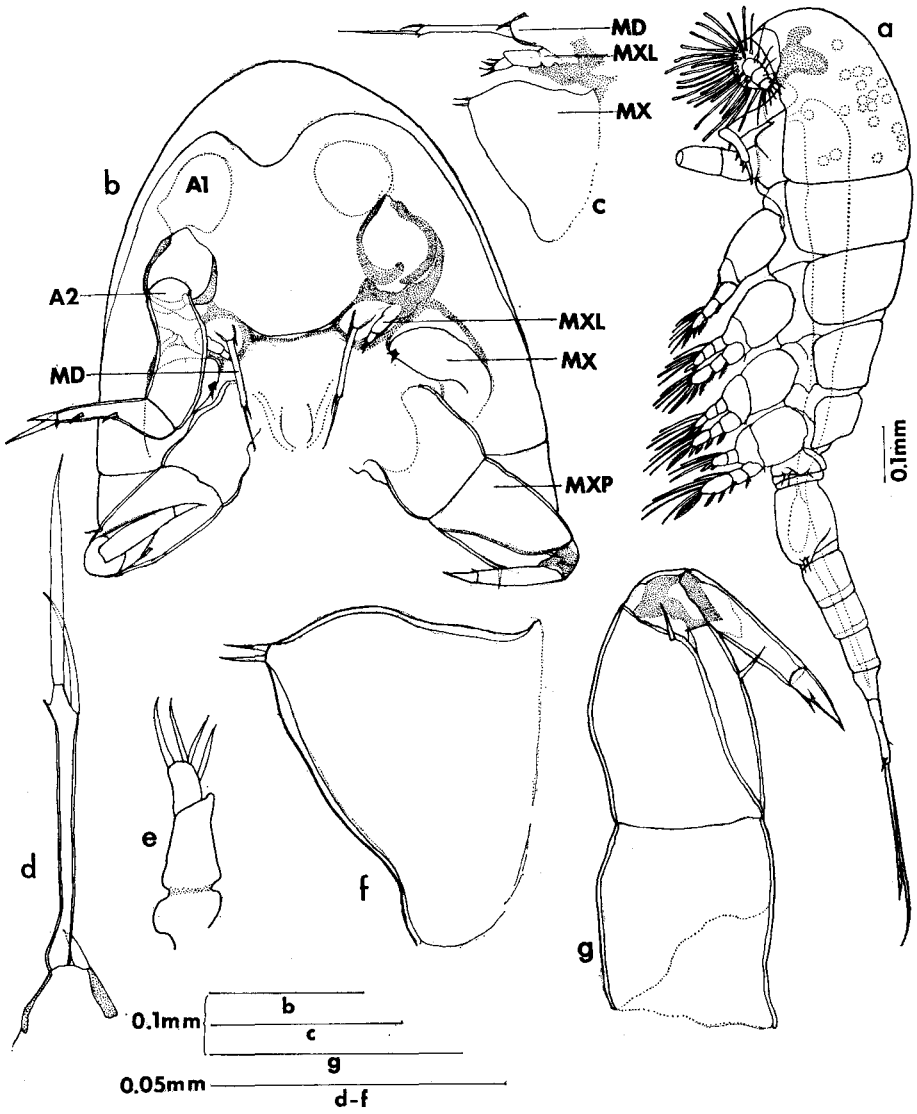

Fig. 8. An unidentified haplostomin, male; a, lateral; b, oral area, ventral; c, mouthparts, lateral; d, mandible (left); e, maxillule (left); f, maxilla (left); g, maxilliped (left).

are composed of the mandibles, maxillae and maxillipeds.

The maxilla of the present male and *Haplostoma* males is comparable with the maxilla of both males and females of *Haplostomella*. In all these forms, the maxilla consists of a large conical lobe with one (in general) apical setule or seta, retaining the basic structure of the swimming copepodid maxilla which is known for *Haplosaccus elongatus*. In comparison, the appendage in the adults is generally more developed in *Haplostomella* than in *Haplostoma* and the same condition is found in the swimming copepodids of these two genera.

In the female of *Haplostoma elegans* a small unsegmented lobe is present between the mandible and the maxilliped. The appendage compares with the maxilla of females of *Haplostomides* in appearance, size and situation, so that it can be designated as the maxilla.

A great reduction of the mouthparts of haplostomins is found in most species of *Haplostoma* females where two pairs of mouthparts are absent between the mandible and the maxilliped. As the maxilla of *H. albicatum* is distinctly developed in the swimming copepodids the degeneration of the appendage in the *Haplostoma* females probably takes place after the swimming copepodid stages.

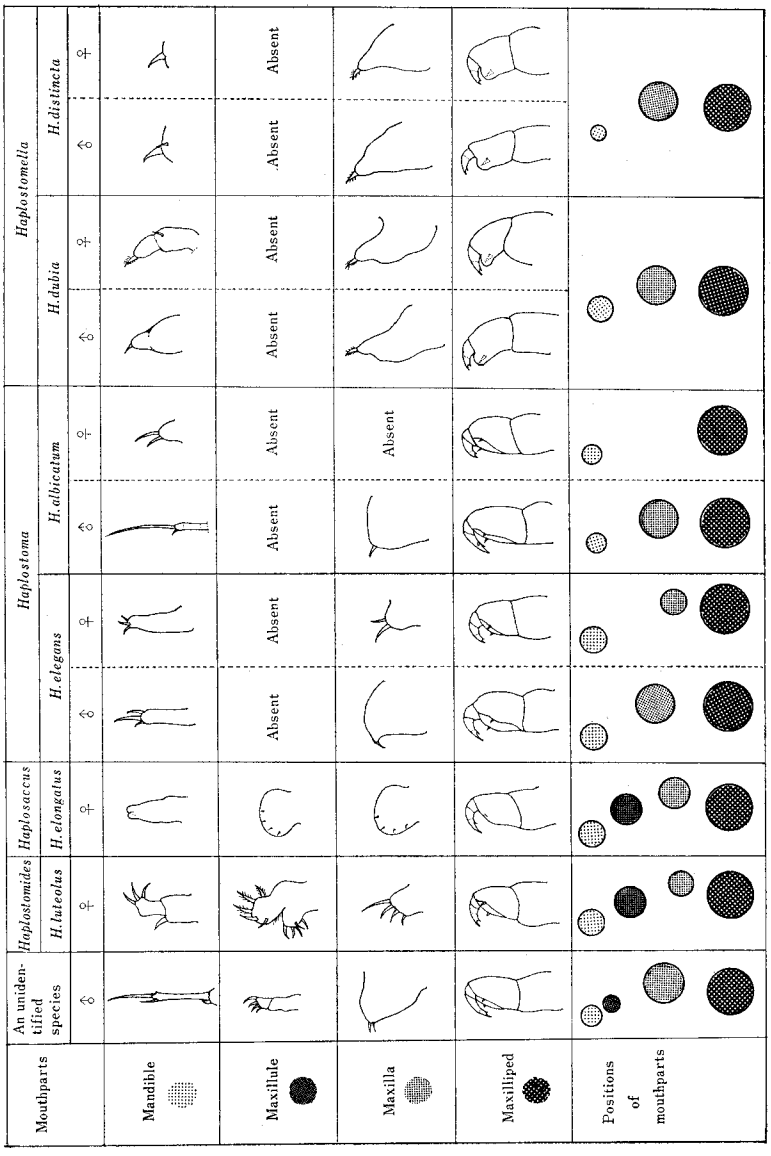

Fig. 9. Morphological comparison of the mouthparts (left, posterior view) in selected haplostomin species.

The constitution of the mouthparts in the subfamily Haplostominae from North America is outlined in Figure 9, which includes some new or revised terminology for the mouthparts of *Haplosaccus, Haplostoma* and *Haplostomella,* and also shows the relative positions and relative sizes of the mouthparts in the males and/or females of the known four genera; in addition the mouthparts of the unidentified male are included.

Discussion

The duration of development of the embryos from laying to hatching in *Haplostomella distincta,* 17 days, is in good agreement with most of the copepods associated with ascidians, such as an ascidicolin (18 days in *Ascidcola rosea*) examined by Gotto (1957, p. 288), an endoparasite in an ascidian (20 days in *Gonophysema gullmarensis*) by Bresciani and Lützen (1961, p. 353), and some notodelphyids (14–15 days in *Doropygus seclusus*; 16 days in *Doropygopsis longicauda*; 16–18 days in *Pygodelphys aquilonaris*) by Dudley (1966, p. 16). Although I could not confirm the exact durations of the embryonic stages of all of the North American species, it is considered that those of other genera, *Haplosaccus* and *Haplostoma,* could be comparable to the above-mentioned ascidicole copepods, because their very young egg masses, obtained directly from their host ascidians, hatched after culturing for 14 (in *Haplosaccus elongatus*) or 15 (in *Haplostoma minutum*) days.

Anderson and Rossiter (1969, p. 466) have estimated that the embryonic period of *Haplostomella australiensis* is 6–8 weeks, which compares to that of many parasitic copepods (Caligidae) on fishes reported by Wilson (1905, p. 535; 1907a, p. 684; 1907b, p. 333). According to Anderson and Rossiter (p. 472), the extended embryonic development of these parasitic copepods is a reflection of a combination of larger eggs and prolonged development to a late larval stage before hatching. However, their opinion seems to be open to question in this case, because the egg size of *H. australiensis* (0.2 mm in length) is not unusually large in comparison with that of the North American species.

In the larval developmental studies on *Haplostoma brevicauda,* Canu (1892, pp. 88–90, pl. XXI, figs. 1–6) has shown three naupliar stages for the species. In comparison of the nauplii in these three stages with the first to fifth nauplii of *H. albicatum,* the nauplii (without postmandibular appendages) in the first two stages of *H. brevicauda* seem to be comparable to the first and second nauplii of *H. albicatum.* The remaining nauplius, with two subcuticular pairs of mandibles and maxillipeds (second maxilla by Canu) and two subcuticular pairs of legs, of the former species probably corresponds to the fourth or fifth nauplius of the latter species. As the successive and gradual development of the postmandibular appendages during the naupliar stages has been observed in the larvae of *H. albicatum* and *Haplosaccus elongatus,* it can be estimated that at least one or two additional naupliar stages probably occur before the first copepodid stage of *H. brevicauda.*

The first copepodids of both of these *Haplostoma* species (Canu, 1892, p. 90, pl.

XXI, figs. 7–10 for *H. brevicauda*) correspond to each other in having many common morphological features: for instance, variably shaped pigment concentrations within the body, a subconical pointed projection from the oral area, two pairs of swimming legs and a single pair of caudal setae. Therefore, the larval development of *H. brevicauda* is found to conform to Type I in the developmental pattern.

Anderson and Rossiter (1969, pp. 470, 472) have reported that *Haplostomella australiensis* has a much abbreviated larval development, namely its free-swimming larvae comprise only two stages, a single naupliar stage and a single free-swimming copepodid stage, which enters the ascidian host, and the period of the naupliar stage is several hours (2–3 hours from hatching to becoming quiescent and 3–4 hours from quiescence to molting for the first copepodid). *H. distincta* and the other *Haplostomella* species from North America also show an abbreviation of the naupliar larval stages and a similar period (7–14 hours) for these stages. Moreover, the morphological features of the first copepodid of *H. australiensis* resemble those of the first copepodid of the North American species, bearing three pairs of swimming legs. Therefore, the larval development of *H. australiensis* is probably included in Type II, which substantially differs from the developmental pattern (Type I) for *Haplostoma* and *Haplosaccus,* and probably *Haplostomides.*

Concerning the pigment spots of the notodelphyids, Dudley (1966, pp. 24–25, Fig. 8) has given a morphological analysis. It is interesting that the pattern of the pigment spots in the above-mentioned Type II, which principally consists of four pairs of round pigment spots, does not belong to any of the patterns in the notodelphyids, and also that the remarkable abbreviation of some naupliar stages is not found in the larval development of the latter family. In the notodelphyids (Dudley, 1966, p. 21), in which generally five naupliar ecdyses occur, the total span for all the naupliar stages are 4 to 4 1/2 days (in *Notodelphys, Pygodelphys, Doropygopsis* and *Doropygus*). In a notodelphyid *Pachypygus gibber,* which has six naupliar stages, the duration for the naupliar stages is 2 days (Hipeau-Jacquotte, 1978, p. 172). These rather resemble Type I, in which the same or similar number of naupliar ecdyses, similar periods (2 3/4 days in *Haplosaccus elongatus,* 2 2/3 days in *Haplostoma albicatum,* 5 days in *H. elegans*) and a similar pattern of pigment concentrations are found.

However, regarding morphological features of their larvae there exist some distinct differences between the haplostomins including Types I and II and the notodelphyids. In the first nauplius of the haplostomins, the distal article of the antennule lacks an aesthete, which is found in the same appendage of the first nauplius of the notodelphyids studied by Dudley (1966, pp. 32–33) and Hipeau-Jacquotte (1978, p. 157), although Dudley (p. 157) already has pointed out its difference in her detailed comparison of the naupliar appendages of notodelphyids with those of ascidicolids (*Botryllophilis,* "*Aplostoma*", *Enterocola* and *Enteropsis*). In addition, the first copepodid of Type I of the haplostomins corresponds to the first copepodid of the notodelphyids in the segmental composition of the tagmata of the body, but the first urosomal segment in the former group has a seta-like indication of the third leg instead of the lobe-like structure of the third leg in the latter group (Dudley,

1966, p. 68; Hipeau-Jacquotte, 1978, p. 159). The first copepodid of Type II of the haplostomins also does not wholly belong to that of the notodelphyids but corresponds to their second copepodid (Dudley, 1966, p. 68; Hipeau-Jacquotte, 1978, p. 159) in having the metasome with three pairs of swimming legs.

Gonophysema gulmarensis (Bresciani & Lützen, 1961, p. 355) has been reported to have an abbreviation of larval development similar to the case in *H. australiensis,* but its first copepodid definitely deviates from any type of the above-mentioned first copepodid in morphology. The genus probably does not belong to the Notodelphyidae nor the Ascidicolidae.

During the process of rearing the larvae of haplostomins, I obtained some abnormal larvae, such as a non-molting nauplius (*Haplostomides luteolus*) which remained viable in culture for more than three weeks and the abnormally elongated nauplius (*Haplostoma elegans*) in which the structure of the first copepodid was cramped in its naupliar cuticle. Dudley (1966, pp. 52–56) has shown many abnormalities of the larvae of notodelphyids by experiments with metal-contaminated cultures. From her valuable experiments, it is evident that the sea water for the culture was contaminated by a very small amount of metal, and this in turn, caused the present abnormalities. In this case it is considered that the metal had been in contact with the culture dishes holding the sea water and larvae, because I inadvertently used tap water passed through a metal pipe to clean the dishes.

The terminology of the mouthparts for the North American species includes some new or revised terminology for the mouthparts of *Haplostoma, Haplostomella* and *Haplosaccus*. The revised terminology could be applied to most of the other known species (female) of the subfamily for the following reasons.

According to Canu (1892, pp. 89, 90, pl. XXI, figs, 6, 9), in the later naupliar stage of *Haplostoma brevicauda* a rudimentary "first maxilla" (=maxillule) is present between the naupliar mandible and the "second maxilla", but degenerates in the first copepodid. Therefore, the mouthparts of the adult female consist of the mandibles and the "second maxillae". This "first maxilla" of Canu corresponds to the rudimentary maxilla of the fourth or fifth nauplius of *H. albicatum* but it remains in the first copepodid of the latter species. The maxilla in *H. albicatum* degenerates in the female copepodid or adult female, so that the mouthparts of the female are composed of the mandibles and maxillipeds. Dudley (1966, p. 160) already has pointed out that the "second maxilla" by Canu is the maxilliped in an "*Aplostoma*" (=*Haplostoma*) species she examined. As in the case of the "*Aplostoma*", the "second maxilla" of Canu also corresponds to the maxilliped in the larvae and adult of *H. albicatum*. Therefore, the terminology which Canu used for the "second maxilla" does not agree with the present terminology, which is in agreement with Dudley's opinion.

In "*Aplostoma*" sp., Dudley (1966, p. 160) reported that the mandible shows great reduction at the first copepodid stage and later disappears, the maxillule is reduced, and the maxilla never forms. In this view, therefore, the mouthparts of the first copepodid are composed of the maxillules and maxillipeds, rather than

mandibles and maxillipeds. Be that as it may, the morphogenesis from the naupliar mandible to the minute copepodid mandible has been examined in *H. albicatum*. This morphogenesis is comparable to what Canu observed in *H. brevicauda* (Canu, 1892, p. 90, pl. XXI, figs. 10, 11), and the tiny mandible with 2 short setae in its first copepodid remains in the adult female (Canu, 1892, p. 223, pl. XX, figs. 8, 9) as seen in *H. albicatum*. In view of these facts we can safely say that the mouthparts of *Haplostoma* females, where two pairs of mouthparts are absent, generally consist of the mandibles and maxillipeds. The same terminology for the mouthparts of the so far known species is found in *H. canui* (Chatton and Harant, 1924c) and *H. gibberum* (Shellenberg, 1922).

In *H. eruca* (Norman, 1869) the appendages of the oral area were not described in the original paper. In its rediscription by T. and A. Scott (1892) the mandible has been shown as an unbelievable structure (p. 204, pl. XVI, fig. 60). The remaining known *Haplostoma* species, *H. banyulensis* (Brément, 1909) and *H. mizoulei* Monniot, 1962, lack the usual mandible. In these three species, the distinctly developed appendages in the oral area seem to be only the maxillipeds (posterior foot jaws in *H. eruca,* by T. and A. Scott; second maxillae in *H. banyulensis,* by Brément). It seems that among these species *H. eruca* should be restudied in regard to the mouthparts.

The terminology of the mouthparts (mandibles and maxillipeds) which was used for *Haplostomella magellanica* Chatton and Brément, 1910, *H. malacocera* Chatton and Harant, 1924a, *H. sycozoae* (Sarfi, 1926) and *H. australiensis* Gotto, 1970 is in disagreement with that for the species of North American *Haplostomella* and for *H. halocynthiae*. The large "mandible" of the former four species apparently corresponds to the maxilla of the latter group of *Haplostomella* species. It is probable that in the former species the presence of the minute spiniform mandible characteristic for the North American species (*H. distincta*; *H. oceanica*) and for *H. halocynthiae* was overlooked by the previous authors. If not so, the mandible in the former species could be indistinct as seen in *H. reducta* from North America.

The mouthparts of *Haplostomella tuberculata* Chatton & Harant, 1924a were reported to consist of the mandibles, maxillules and maxillipeds. The mandible of this species is not spiniform but a palp-like structure with a small apical setule. Such a mandible is found in *H. dubia* from North America. However, the "maxillule" in the former species is comparable to the maxilla of the latter species and also for all other species of North American *Haplostomella* and *H. halocynthiae*. Therefore, the terminology for the mouthparts of *H. tuberculata* perhaps should be revised as the mandibles, maxillae and maxillipeds. It is noted that the appearance and structure of the bodies of *H. tuberculata* and *H. dubia* closely resemble each other, differing somewhat from other *Haplostomella* species.

The presence of the full set of mouthparts, though in extremely modified forms, in the female of *Haplosaccus elongatus* has been recognized by the present study of its larval mouthparts. *H. sacculus,* where no mouthparts except for the maxillipeds were described by Chatton and Brément (1910), probably would present a similar mouth-

part complement in its adult female in reexamination.

The females of *Haplostomides* possess all mouthparts in more developed forms. In general, the setose and bimerous (or biramous by some authors) maxillule is large in size and much more developed than the small maxilla in reduced forms. This mouthpart pattern is found in the female of *H. amarouci* (Blake, 1929), *H. scotti* Chatton and Harant, 1924b, *H. brementi* Chatton and Harant, 1924b and also the North American species. In *H. hibernicus* (Chatton and Brément, 1910), however, the morphology regarding the two mouthparts does not belong to the above-mentioned pattern but resembles the case in the male of the unidentified haplostomin, which has been useful in designating the missing mouthparts of the North American haplostomins. In *H. hibernicus,* the unsegmented maxullule with 4 short non-plumose setae is smaller than the large conical maxilla with 1 terminal and 2 outer similar setae. Although the maxillule of *H. hibernicus* is better developed than the maxillule of the male in question, the armature is the same in both these species. The maxilla of *H. hibernicus* also closely resembles the conical maxilla with 2 terminal setae of this male. These facts suggest that the mouthparts of the unidentified male species do not extremely deviate from those of the species of *Haplostomides* in morphology.

However, more study on the mouthparts of the actual males and larvae (in entire copepodid stages) of *Haplostomides* and *Haplosaccus* is definitely needed to disclose the identity of the unidentified male species and to reinforce the rationale for the terminology used for the mouthparts of North American species. Similar study on botryllophilins, which are the most closely related to haplostomins among the several groups of the Ascidicolidae, would be useful for the same purpose.

Anderson and Rossiter (1969, p. 472) stated that in *Haplostomella australiensis* the first swimming copepodid shows sexual dimorphism. The male can be distinguished from the female by the antennules, which are segmented in the male and small and inconspicuous in the female, and by the remaining naupliar limbs and the thoracic legs, which are larger and more setose in the male than in the female. However, the present study has not brought forth any first copepodid which exhibited sexual dimorphic features as observed in *H. australiensis.*

There are some confusing aspects in minor sexually dimorphic features occurring in certain stages of the later copepodids of *H. distincta.* The third copepodid of this species exhibited the male mandible in spite of its female-like vermiform body. A similarly vermiform copepodid, probably in the fourth copepodid stage, possessed the female type of mandible, but the four-segmented urosome and apparently segmented sclerotizations in the legs seemed to show male features. Distinct sexual characters were evident in the probable fifth copepodids of *H. oceanica* and *H. dubia.*

As the first aim of the present investigation was not concerned with the sexual dimorphism, observation on this problem may not have been consistent. Therefore, the number of examples of copepodids applied to the study on the problem was not sufficient. However, these examples referred to above suggest that there are changes in copepodid morphology at various stages and some dimorphic features, such as antennules, mandibles, urosome, caudal setae, etc., may be unstable in this

group.

Although it is difficult to determine which copepodid stages manifest sexual dimorphism, in the present material of haplostomins the distinct dimorphism seems to occur in the later copepodid stages as seen in the larval development of the Notodelphyidae studied first by Dudley (1966). According to her excellent studies, the dimorphic features occur in the fifth copepodid stage in *Notodelphys, Pygodelphys* and *Doropygus* and in the fourth copepodid stage in *Scolecodes* (p. 139). Recently Hipeau-Jacquotte (1978) has presented the same result in *Pachypygus* of the same group. It would seem very possible that some of the differences between males and females of haplostomins are achieved gradually through a series of copepodid stages and even the copepodids of males can emerge as adults after one or a series of copepodid stages in creeping form.

This study of the larval development of haplostomins is incomplete, with the lack of detailed descriptions of exuviae in each larval stage and also the failure in culturing and in sampling of a complete series of larvae of each genus. However, in the light of our taxonomic paper on the North American haplostomins (Ooishi and Illg, 1977), it has been considered necessary to present this study to give the rationale for the terminology of their mouthparts.

REFERENCES CITED

Anderson, D.T. & G.T. Rossiter, 1969. Hatching and larval development of *Haplostomella australiensis* Gotto (Copepoda, Fam. Ascidicolidae), a parasite of the ascidian *Styela etheridgii* Herdman. Proc. Linn. Soc., New South Wales, 93(3): 464–475.

Brément, E., 1909. Contribution à l'etude des copépodes ascidicoles du Golfe du Lion. Arch. Zool. Expér. Gén., sér. 5, 1 (Notes & Rev.): 61–89.

Bresciani, J. & J. Lützen, 1961. *Gonophysema gullmarensis* (Copepoda parasitica). An anatomical and biological study of an endoparasite living in the ascidian *Ascidiella aspersa*. II. Biology and development. Cah. Biol. mar., 2: 347–372, pls. 1–2.

Canu, E., 1886. Description de deux copépodes nouveaux parasites des Synascidies. Bull. Sci. France-Belgique, ser. 2, 17: 309–320, pls. 2–3.

————, 1892. Les copépodes du Boulonnais: Morphologie, embryologie, taxonomie. Trav. Lab. Zool. Marit. Wimereux-Ambleteuse, 6: 1–354, pls. 1–30.

Chatton, E. & E. Brément, 1910. Sur trois ascidicoles du genre *Aplostoma* Canu: *Aplostoma magellanica* n. sp., *A. hibernica* (T. & A. Scott), *A. sacculus* n. sp., Bull. Soc. Zool. France, 35: 80–92.

Chatton, E. & H. Harant, 1924a. Notes sur les copépodes ascidicoles. XVI.-Le nouveau genre *Haplostomella*. Deux espèces nouvelles de ce genre. Remarques sur les oostégites et la 5e paire de péréiopodes. Bull. Soc. Zool. France, 49: 398–406.

————, 1924b. Notes sur les copépodes ascidicoles. XVII.-Le nouveau genre *Haplotsomides* et deux espèces nouvelles de ce genre: *Haplostomides scotti* et *Haplostomides brementi*. Bull. Soc. Zool. France, 49: 406–412.

————, 1924c. Notes sur les copépodes ascidicoles. XVIII.-*Haplostoma canui* n. sp.-Etat actuel de la systématique des Haplostominae n. subf.-Le nouveau genre *Haplosaccus*. Bull. Soc. Zool. France, 49: 413–422.

Dudley, P.L., 1966. Development and systematics of some Pacific marine symbiotic copepods. A study of the biology of the Notodelphyidae, associates of ascidians. Univ. Wash. Publ. Biol., 21: i-v+1-282, pls. 1–51.

Gotto, R.V., 1957. The biology of a commensal copepod, *Ascidicola rosea* Thorell, in the ascidian *Corella parallelogramma* (Müller). J. Mar. Biol. Assoc., U.K., 36: 281–290.

————, 1970. *Haplostomella australiensis* n. sp., an ascidicolous copepod from New South Wales. Crustaceana, 19(3): 267–272.

Hipeau-Jacquotte, R. 1978. Dévelopment post-embryonnaire du copépode ascidicole Notodelphyidae *Pachypygus gibber* (Thorell, 1859). Crustaceana 34(2): 155–194.

Monniot, C., 1962. *Haplostoma mizoulei* n. sp., copépode parasitic d'une ascidie interstitielle. Bull. Soc. Zool. France, 87(5–6): 570–574.

Norman, A.M., 1869. Shetland final dredging report. Part II. On the Crustacea, Tunicata, Polyzoa, Echinodermata, Actinozoa, Hydrozoa and Porifera. Rep. British Assoc. Adv. Sci., 38th meeting, 1868, pp. 247–336.

Ooishi, S. & P.L. Illg, 1974. *Haplostomella halocynthiae* (Fukui), an ascidicolid copepod associated with a simple ascidian, *Halocynthia roretzi* (Drasche), from Japan. Publ. Seto Mar. Biol. Lab., 21 (5/6): 365–375, pl. XI.

————, 1977. Haplostominae (Copepoda, Cyclopoida) associated with compound ascidians from the San Juan Archipelago and vicinity. Spec. Publ. Seto Mar. Biol. Lab., ser. V, pp. 1–154, pl. I.

Salfi, M., 1926. Su un nuovo copepoda ascidicolo del genere *Aplostoma* Canu. Ann. Mus. Zool. R. Univ. Napoli, 5(15): 1–4.

Schellenberg, A., 1922. Neue Notodelphyiden des Berliner und Hamburger Museums mit einer Übersicht der ascidienbewohnenden Gattungen und Arten. Mitt. Zool. Mus. Berlin, 10: 219–274; 277–298.

Scott, T. & A., 1892. XXII.-On some new or rare crustacea from the Firth of Forth. Ann. Mag. Nat. Hist. ser. 6, 10: 201–206, pls. 15–16.

Wilson, C.B., 1905. North American parasitic copepods belonging to the family Caligidae. Part 1. The Caliginae. Proc. U.S. Nat. Mus., 28: 479–672.

————, 1907a. North American parasitic copepods belonging to the family Caligidae. Part 2. The Trebinae and Euryphorinae. Proc. U.S. Nat. Mus., 31: 669–720.

————, 1907b. A revision of the Pandarinae and Cecropinae. Proc. U.S. Nat. Mus., 33: 323–490.